THE PERIPHERAL ACTION OF THE AUSTRALIAN SNAKE VENOMS.

3. THE REVERSIBILITY OF THE CURARI-LIKE ACTION.

by

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Arthus (1910) has shown that curarization by cobra venom, in its early stages at least, is reversible by antivenine. It seemed of interest to ascertain whether the curari-like action of the Australian venoms was likewise reversible. For this purpose only two monovalent antivenines were available, those against the venoms of the tiger snake *(Notechis scutatus)* and of the copperhead *(Denisonia superba)*, both of which have been prepared by Dr. F. G. Morgan at the Commonwealth Serum Laboratories.

In Arthus' experiments cobra venom in approximately two lethal doses was injected intravenously into rabbits. Respiratory failure took place within half an hour, and antivenine was injected intravenously immediately or at various periods thereafter, the animals being kept alive by artificial ventilation. In seventeen experiments he observed the return of feeble natural respiratory movements in from 1 to 4 hours, and in six natural respiration was sufficient to maintain life within 2½ to 6 hours after the administration of antivenine.

Method. Of the two venoms used in the experiments recorded in the present paper, that of the copperhead, which like cobra venom contains no thrombin, could be injected intravenously in amounts from 1 to 1½ times the lethal dose and, curarization having been quickly produced, antivenine could be given at no very long interval after the introduction of the venom. Tiger snake venom, on the other hand, contains a powerful thrombin, and to avoid intravascular coagulation, two methods were applied. In the first, the venom was injected subcutaneously overnight in an amount less than one certainly lethal dose, and early the following morning a second subcutaneous dose, not large enough to cause intravascular coagulation, but sufficient to cause failure of respiration within 4 or 5 hours, was administered. In these experiments antivenine could not be given till 20 to 25 hours after the first injection of venom. Experiments more comparable with those of Arthus were made, using tiger snake venom in which more than 99% of the thrombin had been removed by my colleague, H. F. Holden, by adsorption with freshly precipitated barium carbonate. Since there was some loss of "neurotoxin" in this preparation, and since the "neurotoxin" of tiger snake venom acts much more slowly those those of the venoms of the cobra and copperhead, rather large amounts (estimated

as from 7 to 10 lethal doses on the assumption that no loss of "neurotoxin" had occurred) had to be injected intravenously to cause failure of respiration in from half to one hour. Antivenine in sufficient amount was injected intravenously as soon as possible after this event.

In all the experiments the march of symptoms was closely similar, though slower in the group in which tiger snake venom was given subcutaneously. There were dyspnoea, loss of tone in the neck and fore-limbs—the head falling into positions determined solely by the action of gravity; the respirations became predominantly costal, then wholly costal, and finally failed altogether. In the later stages there were cyanosis and "starting movements." At this time, when the heart became slowed, and sometimes before the respiratory movements finally ceased, a tracheal cannula was inserted, and artificial ventilation commenced. It was necessary to avoid any prolonged asphyxia in the experiments with copperhead venom, since the partially asphyxiated heart is extremely sensitive to the action of this venom. Copperhead venom was usually given in divided doses to avoid the profound immediate fall of blood pressure which follows the injection of a large intravenous dose.

In applying artificial ventilation care was taken to avoid insufficient ventilation, manifested by the colour of the blood in the veins of the ears and by the continuation during ventilation of movements of the alae nasi, or hyperventilation, causing prolonged apnoea and delay in the onset of movements of the alae nasi, when artificial respiration was temporarily discontinued. In a few experiments the thorax was opened under ether anaesthesia, when respiration had failed, and the phrenic nerve and diaphragm were tested at intervals after the administration of antivenine, to ascertain if any improvement in the responses took place. At the end of most of the other experiments an anaesthetic was administered, the blood pressure was recorded, and the phrenic nerves and diaphragm were tested by faradic stimuli.

In every case much longer periods of artificial ventilation were necessary than in Arthus' experiments. To avoid alkalaemia, due to slightly excessive ventilation over very prolonged periods, oxygen containing 5% CO_2 was used in some experiments, either from the outset or in the later stages only. The Starling Ideal pump was used, with a Douglas bag as the gas reservoir, which was refilled from cylinders of the gases mixed in the correct proportions. When this mixture was reinspired, a leak was provided in the system so as to avoid the inhalation of a mixture excessively rich in CO_2. The gases were sometimes inhaled dry and sometimes saturated with water at room temperature, and arrangements were made for draining off condensed water from the tube connecting the outlet from the tracheal cannula with the pump.

The animals were kept warm on the Palmer table, and the gases or air supplied for ventilation were warmed when necessary. Particular attention was directed to the posture of the animals, which was changed repeatedly during the course of the longer experiments.

1. EXPERIMENTS WITH COPPERHEAD VENOM INJECTED INTRAVENOUSLY.

In these antivenine was injected a few minutes after failure of respiration, as judged by absence of respiratory movements (apart from those of the alae nasi), when artificial ventilation was discontinued for about a minute. The antivenine contained 0·3% tricresol, added before the final filtration of the serum. This seemed unlikely to produce any serious effect on the animal, since control horse serum, which was kindly prepared for me by Dr. F. G. Morgan, and contained the same amount of tricresol added before filtration, could be administered to normal rabbits in a dose of 40 c.c. intravenously without causing any observable symptoms.

The results of four experiments with copperhead antivenine are set out in Table 1. Antivenine was given a few minutes after failure of respiratory movements was demonstrated. In the table the times to commencement of natural respiratory movement and to the end of the experiment are taken from the moment of this injection. The tests of curarization in the first two were made with the animals under full ether anaesthesia, the heart beating strongly. In the third they were made immediately after the heart had failed. In the last experiment respiration failed finally, after having been satisfactorily established for 1½ hours, and the observations were omitted. In the third experiment a long period of inadequate ventilation, due to the presence of some dried blood in the trachea may have been in part responsible for failure of recovery of respiratory movements. Nearly all the animals treated with copperhead venom suffered from severe haemoglobinuria, and one or two which we have not recorded died a few hours after the injection of antivenine from various causes.

TABLE 1.

Reversibility of Curarization by Copperhead Venom, when Antivenine was administered 1½ to 6 hours after the Initial Dose of Venom.

Weight of rabbit in kgs.	Dose of venom in mgs.	Approximate number of lethal doses of venom.	Time to failure of respiration.	Neutralizing value of antivenine in mg. of venom.	Test of curarization at end of experiment.	Temperature at end of experiment.	Number of hours after antivenine administered before adequate natural breathing commenced.
1·8	0·8	1·5	1¼ hours	1·6	Ph=32 D=20 Sc=11 M=13 (36 hours)	37°C.	19½ hours, for ½-hour and for various periods later.
2·13	0·7	1·1	6 hours	1·6	Ph=35 D=25 Sc=5 M=30 (27½ hours)	36°C.	19⅔ hours, for 6 hours.
2·23	0·8	1·2	1½ hours	1·9	Ph=4 D=18 Sc=0 M=23 (21⅓ hours)	36°C.	No return of diaphragmatic or costal respiratory movement.
2·16	0·8	1·2	1⅔ hours	1·5	—	35°C.	29 hours, for 1½ hours.

Ph=phrenic nerve, D=diaphragm, Sc=sciatic nerve, M=innervated muscles.

Although in none of these experiments was there complete recovery of the limb muscles, and in none was the animal allowed to survive, it is clear that some reversal of curarization by this venom has occurred. Unfortunately it was not possible to try the effects of larger doses of antivenine. With copperhead venom recovery is a more gradual process than Arthus found with cobra venom. The diaphragm recovers before the neck muscles, and these before the

limb muscles. In my experiments the very prolonged period of artificial ventilation, and the necessarily long time during which the animals were without food and water, must have had an extremely adverse influence. When to this is added the difficulty of obtaining a completely satisfactory artificial ventilation for so many hours without the production of either alkalaemia or asphyxia, it is not surprising that the early stages only of the recovery process have been observed.

Attempts were made to provide against dehydration (which was increased by the salivation caused by the venom) by the injection subcutaneously or intravenously of saline, of saline with glucose, and of saline, glucose, and insulin, but these were not strikingly successful.

That some degree of recovery of the diaphragm takes place shortly after the administration of antivenine (though this cannot be observed in the experiments of Table 1) was seen in two experiments in which the thorax was opened, under ether anaesthesia and artificial ventilation, shortly after failure of respiration. In both of these, tests of the irritability of the diaphragm by direct and indirect faradic stimulation showed definite improvement within an hour after antivenine was given.

2. EXPERIMENTS WITH TIGER SNAKE VENOM INJECTED SUBCUTANEOUSLY.

In only one of these experiments was any return of natural respiratory movements noted, though in some of them artificial ventilation was maintained for more than 24 hours. This result was confirmed by final tests of the diaphragm by direct and indirect stimulation which revealed striking loss of irritability.

In Table 2 there are set out the essential data concerning five such experiments. In the third of these the thorax was opened shortly after natural respiratory movements ceased, and during more than eight hours no improvement in the irritability of the diaphragm to direct or indirect stimulation was observed, though enormous doses of antivenine were exhibited. This result is in striking contrast to the early improvement seen in similar experiments with copperhead venom.

The first experiment in the table was only carried on for eight hours, and at the end of this time no improvement in the irritability of the diaphragm was noted. The second experiment was the only one in this series in which the observations on the irritability of the diaphragm were not made during the life of the animal. They were made, however, within a few seconds of cessation of the heart, which failed suddenly.

Careful autopsies showed that there were no thrombi in the heart, great vessels, or lungs of any of these animals. The blood was fluid, and in the two

long experiments only slight lengthening of the clotting time was observed. In the third animal only were there any gross macroscopic haemorrhages in the lungs or elsewhere, due to the action of the venom, though slight haematuria was present in the last two. Only in the fourth experiment was there any suggestion of a slight return to activity of the diaphragm six hours after the injection of antivenine.

TABLE 2.

Irreversibility of Curarization by Tiger Snake Venom when Antivenine was administered more than 20 hours after the Initial Dose of Venom.

Weight of rabbit in kgs.	Total dose of venom in mgs.	Approximate number of lethal doses.	Time from initial injection of venom to failure of respiration.	Time to administration of antivenine.	Neutralizing value of antivenine in mg. of venom.	Test of curarization at end of experiment.	Blood pressure at end of experiment.	Temperature at end of experiment.	Number of hours after administration of antivenine to end of experiment.
			hours.	hours.	mgs.				hours.
1·89	0·157	1·8	25	25½	2·0	Ph=0 D=0 Sc =5 M=18	96 mm. Hg.	35°C.	8
1·87	0·112	1·3	23½	24	5·0	Ph=0 D=0	—	35°C.	8½ (died)
1·78	0·253	3·1	22¼	23 24½	3·0 3·2	Ph=0 D=0	—	35°C.	8¼ (died)
2·32	0·28	2·7	21½	21½	4·8	Ph=0 D=0	96 mm. Hg.	33°C.	26½
2·29	0·17	1·7	19¼	20½	2·7	Ph=7 D=0 Sc =20 M =25 H =0 T=5	102 mm. Hg.	37·3°C.	28½

Ph=phrenic nerve, D=diaphragm, Sc=sciatic nerve, M=innervated muscles, H=hypoglossal nerve, T=tongue.

In Arthus' experiments the time elapsing between failure of respiration and the administration of antivenine did not appear to be important, but shortening of the time required for recovery resulted from increase of the dose of antivenine. Here, despite doses of antivenine, varying from 16 to nearly 50 times that necessary to neutralize the venom *in vitro*, there was no appreciable recovery under the conditions of experiment. It is, however, to be noted that Arthus did not in any experiment delay the administration of antivenine more than four hours after the injection of venom. His experience does not therefore exclude the possibility that *prolonged* unopposed action of venom may have profound effects, not only in producing haemorrhages in the lungs and elsewhere, which militate against survival, but by the direct action of the venom upon muscle, which accompanies curarization. When tested at the end of all these experiments, and in the third experiment in Table 2, immediately after failure of respiratory movement also, the muscle was quite insensitive to direct faradic stimulation,

even with the coils closely approximate. As suggested by Houssay and Pave (1922), a view which is supported by observations in an earlier paper (Kellaway and Holden (1932)), this direct action on muscle is probably due to the action of venom lecithinase in altering the permeability of the cells. It would not be anticipated that such gross alterations in permeability would readily be reversed by antivenine, though it might be expected that the more subtle changes, which result in block of the nervous impulse to the diaphragm, and closely resemble the action of curari itself, would, like this, prove to be reversible.

In experiments upon isolated amphibian tissues the direct action of cobra venom upon muscle was much greater than that of the venom of the copperhead, and the direct action of tiger snake venom was less than that of either (Kellaway and Holden (1932)). When, however in experiments upon rabbits, tiger snake venom was allowed a long time to act, the loss of irritability of muscle was greater than when cobra and copperhead venoms in higher concentrations acted for short periods (Kellaway, Cherry, and Williams (1932)).

The general result of the group of experiments under consideration was particularly disappointing, in view of the success attending the late treatment of a case of tiger snake bite with antivenine by Tisdall and Sewell (1932).

Their patient, who had been bitten 24 hours before admission to hospital, and who had received no effective treatment, had severe "neurotoxic" symptoms. There was bilateral ptosis, the pupils were dilated and fixed, the tongue and jaw muscles and the muscles of the limbs were pareised. Twelve hours after the exhibition of large doses of antivenine the patient commenced to improve, and made an uninterrupted recovery. It is of course possible that a sublethal dose of venom had been injected, and that recovery would have followed without the exhibition of antivenine.

3. EXPERIMENTS WITH A "NEUROTOXIC" FRACTION OF TIGER SNAKE VENOM INJECTED INTRAVENOUSLY.

In these experiments a dry preparation of the active curarizing fraction of this venom, freed from more than 99% of the potent tiger snake thrombin, was used. The results are set out in Table 3. It should be noted that the lethal doses are calculated upon the assumption that there was no loss of the original "neurotoxic" activity of the venom. The actual loss was, however, nearly 50%, so that in the expression of the dosage as mgs. of original venom, and in the lethal doses calculated from these figures, the values given should be halved.

In three of these experiments there was some return of natural diaphragmatic movements after prolonged periods, but the degree of recovery was insufficient to permit of the discontinuance of artificial ventilation.

There was, however, no recovery from the paralysis of the tongue or limbs established before the administration of antivenine, which in all cases was given within a few minutes of failure of respiration in amount nearly ten times that necessary to neutralize the venom present.

TABLE 3.

Reversibility of Curarization by Tiger Snake Venom when Antivenine is given within an hour after the Administration of Venom.

Weight of rabbit in kgs.	Dose of fraction as original venom in mgs.	Approximate number of lethal doses of venom.	Time to failure of respiration.	Neutralizing value of antivenine in mg. of venom.	Test of curarization at end of experiment.	Temperature at end of experiment.	Number of hours after administration of antivenine to onset of breathing.
2·19	·7	7·0	51 minutes	2·9	Ph=9 D=22 Sc=0 M=15 H=0 T=8 (22¾ hours)	34°C.	18¾ hours (feeble and inadequate).
1·94	·75	8·6	40 minutes	3·0	Ph=0 D=9 (23 hours)	35·2°C.	9 hours (for 16 minutes) not quite adequate.
1·82	·6	7·3	45 minutes	2·2	Ph=0 D=9	34°C.	No return of breathing in 18 hours.
1·65	·8	10·8	27 minutes	3·0	Ph=0 D=8 Sc=8 M=14 H=0 T=8 (22 hours)	36°C.	19½ hours, feeble and inadequate.

Ph=phrenic nerve, D=diaphragm, Sc=sciatic nerve, M=innervated muscles, H=hypoglossal nerve, T=tongue.

In none of these animals were any thrombi found in the heart, vessels, or lungs at the end of the experiment, though three of them had severe haemorrhages into the lungs, which may actually have been infarcts. All of them died quite suddenly, the heart, which at one moment was beating strongly, suddenly dilating and ceasing to beat. The observations on the irritability of the diaphragm and skeletal muscles were therefore made immediately after death, and are only of negative value. The loss of direct irritability of the muscles was not quite so striking as in the preceding group of experiments, though the contractions in response to faradic stimuli were so feeble as to be difficult to observe.

Some further short duration experiments were made to ascertain the initial effect of the fractionated venom on the direct and indirect irritability of the diaphragm, and whether this showed any early improvement after the injection of large doses of antivenine.

Shortly after the intravenous injection of venom and before the onset of symptoms, the animals were anaesthetized with ether. A tracheal cannula was tied in, and artificial respiration was commenced so as to avoid any complicating effects of asphyxia. So soon as the thoracic and diaphragmatic respiratory movements failed, as was shown by temporarily discontinuing ventilation, the thorax was opened and the right phrenic nerve and the diaphragm were stimulated with faradic currents. Great care was taken to keep the animals warm, and the skin was drawn up and clipped together with a large paper clip so as to close the thoracotomy

wound between tests. It was found advantageous to record the blood pressure, so as to gain more accurate information as to whether the ventilation with oxygen and 5% CO_2 was adequate or excessive.

Experience in these experiments showed how difficult it really is, without a record of the blood pressure, to be certain that the animal is being satisfactorily ventilated. The sudden deaths observed in the experiments of Table 3 were probably due to insufficient ventilation, dependent in part upon the presence of areas of collapse and haemorrhages in the lungs.

When antivenine was given within two hours after the injection of venom there was striking improvement in the response of the diaphragm to indirect faradic stimuli within the next hour, which was maintained during the succeeding 2 or 3 hours. The response of the diaphragm to direct faradic stimuli did not show corresponding improvement, though natural diaphragmatic respiratory movements could be observed within 4 or 5 hours after the administration of antivenine.

Summarizing the results of all these experiments, it appears that curarization by the venoms of the copperhead and tiger snake is reversible, but that its reversibility is more difficult to demonstrate than is the case with cobra venom, which is more predominantly curari-like in its action. For this reason Arthus was able to administer antivenine earlier after the injection of venom than was possible in my experiments. The added direct action of Australian snake venoms on the muscle of the diaphragm is difficult to reverse when well established; indeed, there is no evidence that it can be reversed within the times covered by these experiments. If long periods of unopposed action are allowed to tiger snake venom, this direct effect on the irritability of muscle becomes fully developed. Short periods of unopposed action of tiger snake and copperhead venoms reveal the reversible curari effect.

ACKNOWLEDGMENTS.

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THE EFFECTS FOLLOWING THYROIDECTOMY IN MERINO SHEEP

by

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The earliest reference to experimental extirpation of the thyroid gland of herbivora seems to be contained in an inaugural dissertation presented at Tübingen in 1840 by v. Rapp, who was unable to detect any untoward symptoms in a goat after removal of the gland. These observations were confirmed some fifty years afterwards by Moussu (1892), and in the meantime Sanquirico and Orecchia (1887) had extended the experimental findings to sheep.

Towards the end of last century, in a summary of the knowledge of thyroid function which was then current, Horsley (1886) divided the various groups of animals into classes which differed markedly in the relative severity of the symptoms supervening on thyroidectomy. He pointed out that removal of the thyroid in *Ungulata* is not followed by the rapidly fatal symptoms of tetany frequently apparent in thyroidectomized carnivors.

Although at this time the parathyroids had been described (Sandström, 1880), no account of them was taken in extirpation experiments, and, in the light of more recent knowledge, it would seem that the acute symptoms which follow the surgical removal of the thyroids from the carnivora could be, for the most part, attributed to the simultaneous removal of the parathyroids, and that the difference in behaviour after thyroid removal might be well explained on anatomical grounds, the close proximity of the thyroid and parathyroids in the carnivors rendering the extirpation of one singularly difficult without simultaneous removal of the other.

In the sheep two parathyroids are embedded in the thymus, one on either side, just above the level of the thyroid cartilage in front of the carotid, while another pair are intimately bound up in the thyroid proper (MacCallum, 1907). In consequence, the removal of the thyroid, with its associated parathyroids, may be accomplished with relative ease, leaving one pair of parathyroids undamaged, and the operated animals evidence no symptoms of parathyroid tetany.

Charcot's description of the syndrome of "cachexie pachydermique" following thyroid insufficiency in humans, with the extensive changes in the skin due to myxoedematous infiltration, the dry, brittle hair, partial alopecia, and altera-

tion in the texture of the nails, might lead one to expect similar far-reaching changes in the integument of thyroidectomized animals, and one might regard as likely that considerable changes, both in the quality and extent of fleece production, would follow thyroid removal from the sheep. There seems still to be considerable controversy in the literature as to the effects of thyroid removal of herbivora.

I. THE EFFECTS FOLLOWING THE REMOVAL OF THE THYROID FROM MATURE MERINO SHEEP.

Lanz (1904) first pointed out that mature animals withstood thyroidectomy better than younger ones, and these findings were extended to sheep by Sutherland Simpson (1924). The symptoms of "ovine myxœdema" are slow

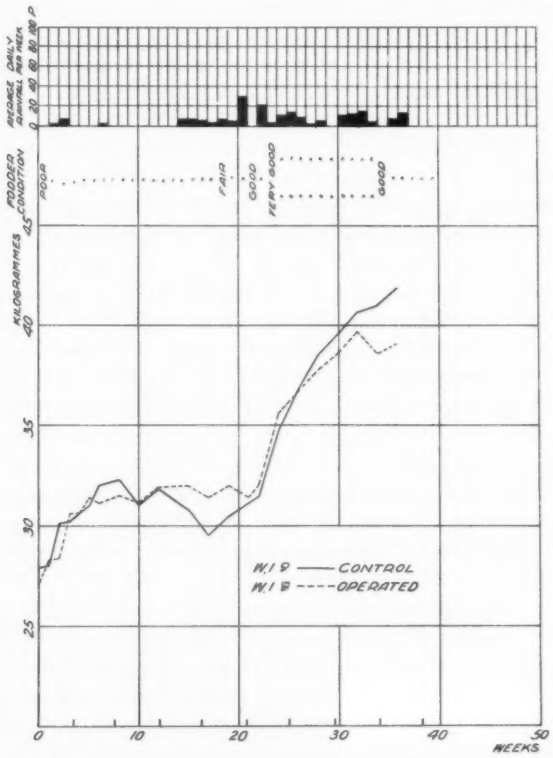


Figure I. The mean growth of thyroidectomized W_1 ewes, operated at one year of age, compared with that of their normal controls. The difference in growth subsequent to the operation is not significant. The operation date, January 5, 1929, is referred to as 0 weeks on the time ordinate.

in appearing, and in our experience, providing the operation is delayed until the animal is about two years of age, the outward signs of thyroidectomy are not at all easily detectable. The basal metabolism of operated animals, however, falls appreciably, sometimes to −40%. Our observations, extending over four years, indicate that the wool growth of such animals was decreased in quantity, while no appreciable difference in fibre diameter was noticed. Thyroidectomized mature ewes carried lambs to full term, and delivered them healthy and normal. Such lambs rarely survived if left with their mothers, as the milk supply of the ewe was insufficient. Figure I represents the mean body weight of a small group of thyroidectomized ewes operated on at about 12 months of age, and their controls, and shows that no very appreciable effect is noticeable on subsequent growth.

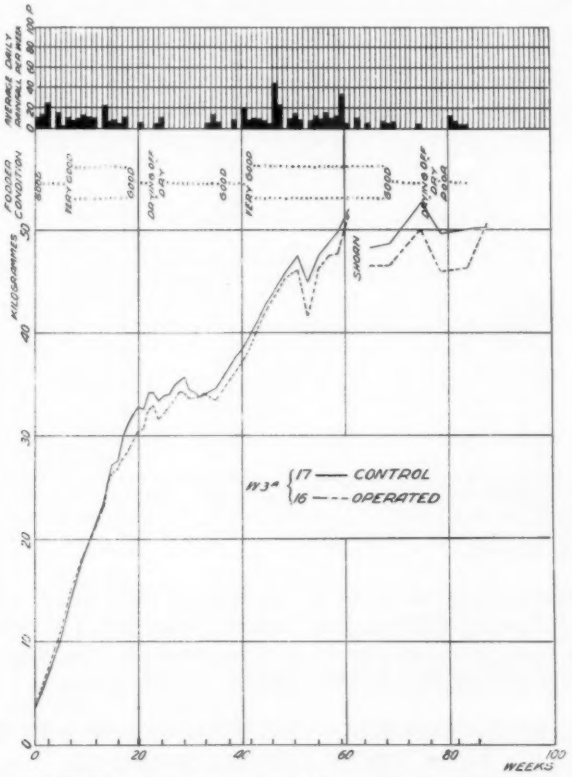


Figure II. The growth curve of ewe W_3A16, which was thyroidectomized at eleven weeks, is contrasted with that of its normal twin control. No significant difference is apparent. At autopsy about 18 months subsequent to operation, about 1 grm. of regenerated thyroid tissue was taken from W_3A16. The weeks on the time ordinate are subsequent to July 14, 1930.

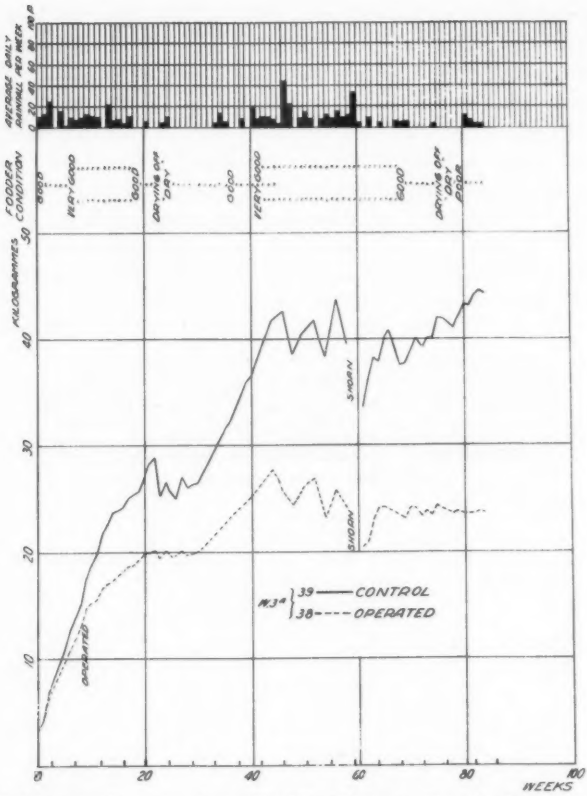


Figure III. The growth curve of ewe W_3A38, which was thyroideetomized at seven weeks of age, is contrasted with that of its normal twin control. W_3A38 (see Figure IV) took on the typical conformation of a cretin. At autopsy, about 18 months subsequent to operation, only a small trace of regenerated thyroid was discernible. The weeks on the time ordinate are subsequent to July 14, 1930.

although the operated animals seemingly maintained their weight a little better during the poor feeding conditions experienced in the period March-May, 1929. All of the animals in these experiments were pastured with the main flock at the Waite Agricultural Research Institute, Adelaide, South Australia.

II. THE EFFECTS FOLLOWING THE REMOVAL OF THE THYROID FROM YOUNG MERINO SHEEP.

In September, 1930, the thyroids were removed from one of each pair of sixteen identical twin lambs, aged between six and eleven weeks. The operations, which were performed under light ether anaesthesia, were uneventful, the lambs

rejoined their mother ewes and fed normally the same day. All wounds healed rapidly by first intention.

TABLE 1.

Basal Metabolism of Operated and Control W_3A. Lambs.

Group W_3A Animal No.	Age when determined (days).	Weight (kg.).	Surface Area (sq. metres).	R.Q.	Cal/m²/hr.	Standard metabolism % of control.
4 ♂ (operated)	126	24·0	0·76	·78	35·4	—43
5 ♂ (control)	143	24·1	0·76	·74	62·2	
14 ♂ (operated)	130	25·5	0·79	·75	52·7	—11
15 ♂ (control)	130	25·0	0·78	·74	58·9	
16 ♀ (operated)	132	25·9	0·80	·76	53·0	—4
17 ♀ (control)	132	27·9	0·84	·76	55·7	
21 ♀ (operated)	123	21·4	0·70	·78	44·2	—20
22 ♀ (control)	141	27·4	0·83	·77	55·8	
24 ♀ (operated)	125	22·9	0·73	·75	52·5	—12
25 ♀ (control)	125	21·3	0·70	·74	59·7	
28 ♀ (operated)	103	20·5	0·68	·79	40·5	—23
29 ♀ (control)	136	22·2	0·72	·77	52·5	
38 ♀ (operated)	105	16·2	0·58	·79	38·1	—32
39 ♀ (control)	105	21·0	0·69	·75	56·5	
24 ♀ (operated) 1*	384	39·8	1·06	·72	44·0	+5
25 ♀ (control) 1*	384	36·0	1·00	·72	42·6	
21 ♀ (operated) 1*	401	34·3	0·97	·73	31·5	—22
22 ♀ (control) 1*	401	40·3	1·07	·73	40·4	
12 ♀ (operated) 1*	408	36·3	1·01	·73	38·8	—3
13 ♀ (control) 1*	408	35·0	0·99	·72	40·0	
28 ♀ (operated) 2*	571	29·8	0·90	·81	30·4	—23
29 ♀ (control) 2*	570	36·8	1·02	·74	39·5	
38 ♀ (operated) 2*	562	22·6	0·76	·79	28·1	—36
39 ♀ (control) 2*	556	36·3	1·01	·74	43·7	
12 ♀ (operated) 1†	424	30·5	0·91	·71	39·6	+10
13 ♀ (control) 1†	424	29·1	0·88	·71	35·2	
21 ♀ (operated) 1†	416	27·7	0·86	·70	32·7	—8
22 ♀ (control) 1†	416	35·7	1·00	·74	35·6	
24 ♀ (operated) 1†	412	31·9	0·93	·74	30·6	—4
25 ♀ (control) 1†	412	32·3	0·94	·71	31·8	
28 ♀ (operated) 2†	487	30·3	0·90	·74	32·9	—28
29 ♀ (control) 2†	486	37·1	1·02	·76	45·7	
38 ♀ (operated) 2†	471	21·7	0·74	·78	28·0	—35
39 ♀ (control) 2†	472	25·6	0·99	·73	43·4	

W_3A 13 was not determined in the early series owing to restlessness which a large dose of 0·8 grm. amytal per os did not affect. In consequence the basal rate of W_3A 12, its operated twin, was not determined at this time.

Note: The above determinations were carried out after 48 hours starvation, the animals lying down.

1* The feed consumed by these animals for some weeks prior to the determination had a nutritive ratio of 1 : 4·3; no record was taken of the amount eaten.

2* The feed consumed by these animals for some weeks prior to the determination had a nutritive ratio of 1 : 3·3; the daily ration containing 1,370 calories was all eaten.

1† The feed consumed for some weeks previous to the estimation of basal metabolic rate had a nutritive ratio of 1 : 20; no record was taken of the amount later.

2† The feed consumed by these animals for some weeks previous to the estimation had a nutritive ratio of 1 : 8·6; the daily ration containing 1,385 calories was all eaten.

(a) *The Effects on Growth Rate.* The mean growth rate of the operated group was decreased, but individual animals differed markedly in the response to the operation. Some of which W_3A. 16 (fig. 2) is a type example, exhibited little or no obvious slowing of growth rate as a result of the operation, while others (W_3A. 38 in fig. 3 for instance) ceased to grow, and took on the conformation of typical cretins. Owing to the exigencies of space the growth data is not presented here, but the relative weights of the operated animals and their controls at different times may be gathered from the tables referring to wool production and basal metabolism.

(b) *The Effects on Basal (Standard) Metabolism.* The standard metabolism of the operated animals and their twin controls was determined at approximately one month and twelve months after operation, by the open chamber method described by Lines and Peirce (1930), and the figures in Table 1 refer to the energy consumption of the quietly resting animal, which has fasted for 48 hours previously, and is free from the thermal influence of cold. The extent of the depression of the basal metabolic rate soon after thyroidectomy varied in the series from —4% to —43%. About a year afterwards the basal rate of some of

TABLE 2.

Production of Wool Fleece by Thyroidectomized Lambs and their Twin Controls during the Year, from 6th to 18th Months of Age.

Group W_3A Animal No.	Weight When Shorn (kgs.).	Total Greasy Wool (kgs.).	Fleece Quality.	Fleece Yield %.	[1] Total Clean Scoured Wool (kgs.).	[2] Clean Scoured Wool per sq. metre surface (kgs.).	Ratio T:C Clean Scoured Wool per sq. metre.	Ratio T:C Total Clean Scoured Wool.
12 ♀ (operated)	40·5	4·20	60	58	2·14	2·00	105	109
13 ♀ (control)	37·8	3·83	60	60	1·97	1·91		
14 ♂ (operated)	43·5	3·44	64/70	72	2·23	1·99	91	93
15 ♂ (control)	42·1	4·17	64/70	66	2·40	2·19		
16 ♀ (operated)	51·4	4·76	60	56	2·41	2·19	107	100
17 ♀ (control)	52·0	5·46	60	50	2·40	2·04		
21 ♀ (operated)	41·8	2·61	60	60	1·36	1·33	60	55
22 ♀ (control)	39·9	4·48	60	56	2·49	2·20		
24 ♀ (operated)	39·0	4·03	58	64	2·21	1·99	95	97
25 ♀ (control)	36·2	4·05	60	58	2·14	2·10		
28 ♀ (operated)	36·7	2·64	64	65	1·59	1·63	76	67
29 ♀ (control)	46·3	5·05	60	56	2·39	2·15		
38 ♀ (operated)	25·9	1·93	64	72	1·20	1·41	72	55
39 ♀ (control)	43·8	5·24	60	48	2·18	1·97		

Note: Lamb W_3A 4 was taken by dogs early in the experiment.

[1] The total clean scoured wool is the amount calculated for the whole fleece by summing the estimated clean scoured wool from the various fractions of the shorn fleece. The figures under "Fleece Yield" refer to the percentage clean scoured wool in the skirted fleece.

[2] The skin areas calculated from body weights.

Ratio T : C expresses production of operated animal in per cent. of that of its twin control.

TABLE 3.

The Skin Areas and Total Clean Scoured Wool Removed from Thyroidectomized and Twin Control Lambs at Autopsy.

Group W_3A. Animal No.	Body Weight. (k.g).	Skin Area (directly measured). sq. metres.	Total Clean Scoured Wool (directly estimated). (kg.).	Wool per Square Metre Surface. (kg.).
12 ♀ (operated)	42·5	1·101	2·305	2·38
13 ♀ (control)	47·8	1·154	2·090	1·81
14 ♂ (operated)	54·7	1·305	2·040	1·54
15 ♂ (control)	63·5	1·322	2·255	1·70
16 ♀ (operated)	50·8	1·211	1·930	1·59
17 ♀ (control)	49·7	1·205	1·962	1·63
21 ♀ (operated)	43·9	1·086	1·396	1·28
22 ♀ (control)	47·1	1·107	1·943	1·76
24 ♀ (operated)	47·8	1·125	1·880	1·67
25 ♀ (control)	46·8	1·130	1·520	1·34
28 ♀ (operated)	33·1	0·890	0·890	1·29
29 ♀ (control)	45·6	1·090	1·150	2·20
38 ♀ (operated)	23·9	0·689	0·700	1·01
39 ♀ (control)	46·1	1·140	2·625	2·31

the operated animals had increased to nearly that of their twin controls, while others continued to evidence a depressed energy metabolism. These latter animals, of which W_3A. 38 (fig. 4) was one, were typical cretins, and exhibited the chronic cachexia, lethargic stupidity, and mental dullness usually associated with this condition.

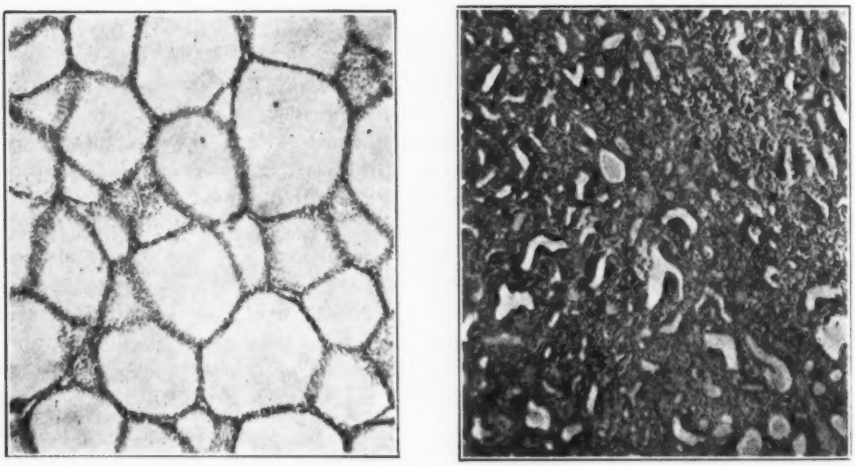
(c) *The Effect on Wool Growth.* The operated animals and their twin controls were shorn when approximately 12 months of age, the wool fleece taken and treated in a similar fashion to that previously described (Marston, 1930).

The estimated amount of clean scoured fibre taken at shearing (Table 2) varied in a similar manner to the depression of growth and of energy metabolism. The condition (greasiness) of the fleece of the operated animals was, in all cases, less than that of their twin controls. No great difference was appreciable in the fibre diameter. The difference in weight of clean wool was thus accountable in most cases by a difference in staple length.

At approximately 20 months of age the animals were killed with chloroform and their skins removed, the areas directly determined by the planimeter, and the wool completely recovered by the fellmongering process of plucking the wool after painting the back of the skin twelve hours previously with 10% sodium sulphide.

The plucked wool was thoroughly scoured, and the clean fibre determined after drying to constant weight *in vacuo* at 100°C. The results of this latter procedure are reported in Table 3. No marked shedding of wool was detected even in those animals which suffered most from the operation.

Figure IV. W_3A39 and W_3A38, twin merino ewes, photographed twelve months after removal of the thyroid from the latter. The dwarfed appearance and cretinoid expression of the operated animal is obvious. The amount of regenerated thyroid in this animal weighed about 0·2 grms., while the normal thyroid of its twin control weighed 3·2 grms.



A B

Figure V. (B) Section of the thyroid residue taken from lamb W_3A38 contrasted with (A) the section from the thyroid of its normal twin control. (Identical magnification.)

(d) *The Regeneration of Thyroid Tissue in Young Lambs.* At autopsy the cause of the varied response to removal of the thyroids became apparent. Thyroid tissue was found in the cervical region of the operated animals, and the amount present could be directly correlated with the measure of effect suffered from the operation, whether this be judged from diminution of growth rate, of basal metabolism, or of fleece production. The regenerated glandular tissue was found mainly in two sites, one, the most frequent, around the base of the inferior thyroid artery about 4 cms. below the normal position of the gland, the other in the cicatrized tissue in the position originally taken by the thyroid ishmus. In one animal ($W_3A.$ 16) a perfectly formed lobe of normal size was found attached to the inferior thyroid artery about 8 cms. below the cricoid cartilage.

TABLE 4.

Dry Weight and Iodine Content of Glands Removed at Operation.

Animal No. $W_3A.$	Age When Operated. Days.	Dry Weight. grm.	Iodine Total. grms.	Iodine % of Dry Weight.
4	84	·443	2·61	·59
12	83	·299	1·19	·40
14	81	·531	2·72	·51
16	80	·444	1·69	·38
21	80	·386	1·71	·44
24	75	·430	·92	·21
28	74	·522	3·50	·67
38	52	·294	5·13	·95

TABLE 5.

Weight and Iodine Content of Thyroid Tissue taken at Autopsy from the $W_3A.$ Series.

Gland. from No.	Total Wet Weight. grms.	Total Dry Weight. grms.	Fraction* used for analysis.	Total Iodine.	Per cent. I. Of Dry Weight.	Per cent. I. Of Wet Weight.
W_3A 12	1·01	·339	·77	1·13	·334	·113
13	2·39	·728	·91	3·24	·445	·136
14	·43	·178	·81	·26	·149	·061
15	2·31	·759	·90	3·83	·505	·166
16	·99	·363	·76	1·53	·421	·154
17	2·36	·746	·93	3·70	·496	·157
21	·58	·172	·78	·80	·467	·138
22	2·07	·668	·90	3·12	·466	·150
24	1·90	·336	·80	·58	·174	·031
25	5·77	1·546	·84	6·29	·407	·109
28	·43	·129	·75	·47	·364	·109
29	2·71	·871	·91	4·42	·508	·164
38	·20	—	nil	—	—	—
39	3·21	1·186	·91	4·34	·366	·135

* A fraction of the tissue was taken for histological examination, and the remainder utilized for analysis.

The tissue taken from the operated animals had, for the most part, regenerated subsequent to thyroidectomy, the operation being performed about eighteen months prior to autopsy.

The weight and iodine content of the removed glandular residues taken at the original operation and at *post mortem* is presented in Tables 4 and 5.

The histological appearance of the glandular residues, when they were small, was one of hyperactivity, and the iodine percentage of these was low. Fig 5 contrasts the histological appearance of the thyroid of W_3A. 38 with that of W_3A. 39, the normal control twin.

The behaviour of W_3A. 16 throughout the experiment would suggest that rather less than half the glandular tissue usually present in the sheep is sufficient to take over full function, for there was no significant difference between the behaviour of this animal and its twin control during the 20 odd months of observation, while the thyroid taken at autopsy, although less than half that of its control, showed no difference either in iodine percentage or in microscopic structure.

DISCUSSION.

It will be seen then that while the appearance of mature merino sheep suffers little obvious change after removal of their thyroids, their basal metabolic rate becomes markedly retarded, and the amount of wool fleece grown is decreased.

The young lamb has the capacity to regenerate thyroid tissue presumably from small glandular residues unavoidably left at operation. This tendency was not shared equally among the lambs, as the weight of thyroid tissue recovered at *post mortem* varied some hundreds of per cent.

The above facts may account for the discrepancies of behaviour following thyroidectomy that have been discussed by other investigators (Gley (1892), v. Eiselsberg (1895), Zietschmann (1907), Sutherland Simpson (1913-1924), Krizenecky (1932)).

When the amount of thyroid tissue is reduced to about one-eighth of the normal the follicles become small, the secretory epithelium is thrown up into folds, and little or no colloid is visible. The iodine content of such glands is low.

To sum up, then, while mature sheep suffered little untoward effect after removal of the thyroids, young animals became cretinized if they failed to regenerate sufficient gland to supply the necessary hormone.

The symptoms of early thyroid insufficiency in sheep are not widely different from those which appear in humans. Thyroidectomized lambs have a reduced basal metabolism. They fail to grow normally and develop the lethargic stupidity of the human cretin. The production of wool is reduced considerably, without evident decrease in fibre diameter, and the degree of greasiness of the fleece is definitely lessened. No symptoms of oedematous infiltration of the integument were observed in any of the operated animals.

ACKNOWLEDGMENTS.

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A PRELIMINARY NOTE ON THE ORIGIN OF A B-TYPE SPELTOID IN *TRITICUM VULGARE*

by

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The occurrence of spelt-like plants in pure strains and hybrid progenies of *Triticum vulgare* has been recorded and investigated by various workers. An adequate review and critique of the literature is given by Watkins (1930), so that only work of immediate application need be referred to here.

ORIGIN OF B–TYPE SPELTOID.

In a cross between two strains of *Triticum vulgare*, Egyptian 8 (♀), an awned white glumed variety, and Hard Federation (♂), an awnless bronze glumed variety, one of the four F_1 plants obtained possessed the spelt characters of lax head, stiff glumes, and strong keel. The normal F_1 plants, when grown the following year, segregated for awns and glume colour in a simple mendelian manner. The F_1 speltoid plant, on the other hand, segregated normally for glume colour, but did not segregate for awns. All the F_2 plants were either tip-awned or almost completely awnless, and 44 were speltoid and 6 normal: a ratio of approximately 7 speltoid : 1 normal.

The ration of 7 speltoid : 1 normal indicates that we are dealing with a B-type speltoid as studied by Nilsson-Ehle (1921). Huskins (1928) has shown that these speltoids have 41 instead of the normal 42 somatic chromosomes. In the speltoid described above it is assumed that spelting and non-segregation for awns is due to the loss of the chromosome carrying the gene for awnedness, which gene was brought into the cross by the female parent Egyptian 8. All the heads produced by the F_1 plant were speltoid, therefore the loss must have occurred at some time prior to the first division of the zygote. The possibility of pollination by stray pollen from a speltoid plant is not a possible explanation, because the female parent was awned, and therefor all F_1 speltoids would be awned.

F_3 BREEDING BEHAVIOUR.

Seeds from each of the 50 F_2 plants were sown as plant to row progenies in F_3. These progenies gave speltoids and normals in proportions varying from 14 : 12 to 29 : 1, with a mean ratio of 4·2 : 1.

Of the 44 speltoid progenies, 8 were homozygous awnless, 25 heterozygous, and 11 homozygous tip-awned. This is a close approximation to a ratio of 11 : 22 : 11 expected on the basis of independent inheritance. That the F_2 segregates tip-awned and awnless independently of spelting and in a simple mendelian ratio suggests that a modifying gene affecting the length of the tip-awn is carried on a chromosome other than the chromosome carrying the gene B_1b_1 for awns.

In a total of 1,060 F_3 speltoid plants examined three were fully awned, weak, and sterile. Such weak, sterile plants are assumed to be homozygous speltoids with 40 somatic chromosomes. That these homozygous speltoids should be awned is explained by the existence of two independent genes for awns. In unpublished data of the senior author it has been shown that by crossing two awnless varieties, e.g., Indian 6 x Gluyas, fully awned segregates appeared in F_2 in the proportion of 15 awnless : 1 awned. The genetic constitution of Indian 6 is shown to be $b_1b_1B_2B_2$, and Gluyas $B_1B_1b_2b_2$. Hybrids between Hard Federation and Gluyas made in 1928 did not segregate awned types in F_2, therefore Hard Federation has the constitution $B_1B_1b_2b_2$. The awnless speltoids then have the constitution $\frac{o}{B_1}\ \frac{b_2}{b_2}$ in which o indicates the missing chromosome. This speltoid is awnless because of the presence of B_1. However, when two 20 chromosome gametes unite to form a homozygous speltoid of the constitution $\frac{o}{o}\ \frac{b_2}{b_2}$ the b_2 gene for awnedness is able to express itself and the plant is awned.

GENETICAL TEST FOR THE ABSENCE OF ONE CHROMOSOME.

A 41 chromosome speltoid plant in which the homologue of the missing chromosome carries the gene for awnlessness, and in which b_2 is homozygous recessive, should, when crossed with an awned non-speltoid plant, give awned speltoids and awnless non-speltoids. In 1931, crosses were made between awnless speltoids (♀) and awned velvet-node (♂) plants. Seventy-nine hybrid seeds were obtained and sown, but owing to severe water-logging of the area only 15 survived; of these, 12 were awned velvet-node speltoid and 3 awnless velvet-node non-speltoid. This, in conjunction with cytological behaviour to be described later, provides good evidence for the loss of one chromosome.

CYTOLOGICAL EXAMINATION OF B-TYPE SPELTOID.

1. *Root Tips.*

Chromosome counts made on polar views of the metaphase stage of division in root tips of three speltoid plants showed 41 chromosomes. Division was quite normal, and no laggards were observed at anaphase. Counts were made from paraffin sections stained with haematoxylin.

2. *Pollen Mother Cells.*

An examination of the maturation divisions of pollen mother cells of six speltoid plants by aceto carmine mounts showed that 21 chromosomes, composed of 20 bivalents and 1 univalent, were regularly present at meiotic metaphase. In many metaphase polar views the univalent was distinguishable on account of its smaller size. Side-view metaphase plates usually exhibited one univalent not lined up on the plate and lying in various positions, most frequently well away from the plate, but sometimes either close to the plate, overlying the plate, or occasionally attaching itself to a bivalent to form a trivalent. Predisjunction of a bivalent was observed in a few cells, but since this irregularity occurs in normal plants no importance is attached to it.

The univalent lagged at heterotypic anaphase in 25% to 50% of the cases, being included in, or excluded from, the daughter nuclei, according to the extent of lagging. In most cases the univalent could be seen to be split at heterotypic anaphase in preparation for the homotypic division. The two halves of the univalent did not usually separate, but moved together to one or other of the poles. Occasionally the split halves of the univalent did separate at heterotypic anaphase, one half passing to each pole. Interphase frequently showed one or, more rarely, two extra nuclei. In the latter case both were usually in one cell, but occasionally one was present in each daughter cell.

Lagging of a univalent was less frequent at homotypic than at heterotypic division. A number of tetrads showed either one or two extra nuclei, sometimes both in the same cell, but more usually in adjacent cells. Thus the univalent seen at meiosis may, in a number of cases, be included in the nuclei of the gametes, or, as is most frequently the case, remain in the cytoplasm, where it degenerates and is lost from the chromosome complement.

Non-speltoid plants from the same lines as the above speltoid plants were examined, and found to contain 21 bivalent chromosomes. No lagging was observed; the only irregularity was an occasional case of predisjunction of bivalent chomosomes.

SUMMARY.

An F_1 speltoid plant, which occurred in the cross Egyptian 8 (♀) awned x Hard Federation (♂) awnless, produced 44 awnless speltoid and 6 awnless normal plants in F_2.

The genetical and cytological evidence presented suggests that the speltoid is of the B-type with 41 somatic chromosomes, that the loss of the chromosome occurred prior to the first division of the hybrid zygote, and that the lost chromosome is the one bearing the gene for awnedness from the Egyptian 8 parent.

A cytological examination of the speltoids shows 41 somatic chromosomes

in root tips and 20 pairs plus 1 lagging univalent at reduction division in pollen mother cells.

A more detailed account of the cytology and breeding behaviour of this speltoid, together with the results of numerous crosses, will be presented in a later paper.

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THE ABSORPTION OF ULTRA-VIOLET RADIATION BY HAEMOGLOBIN AND SOME OF ITS DERIVATIVES

by

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In a previous communication (1929) were recorded the nearer ultra-violet absorption spectra of a number of derivatives of oxyhaemoglobin. The spectrophotometric cells available at that time did not permit the observation of the spectrum of (reduced) haemoglobin at wave lengths less than 3,750 Å.U., owing to the absorption due to the chemicals used for its reduction. In this paper are described the spectra of haemoglobin, reduced haematin ("haem" or "heme"), denatured ox globin haemochromogen, and cyan-haemochromogen. The spectra of all the substances were photographed in the Hicks micrometer cell (1931). Haemoglobin was also studied in another type of cell. This consisted of a tonometer, to which was fused a T-piece, the ends of the arms of which were closed by parallel quartz plates held in position by a screw clamp. The depth of liquid was 0·956 cm. The solution of haemoglobin was placed in the tonometer, and equilibrated several times with hydrogen previously stored for several weeks in contact with an alkaline solution of sodium hydrosulphite. It was transferred, by tilting, to the cell for photography without access of air. The small depth of liquid in the micrometer cell enabled a sufficient amount of sodium hydrosulphite to be used for reduction without its own light absorption obliterating the spectrum. The haemoglobins of the opossum and of the rabbit were used. The difference between them was very slight. The solutions of reduced haematin and of cyan-haemochromogen contained 0·5% gelatine to delay flocculation of the pigment. The control solutions contained every substance in the observed solution except the pigment. The calculations are as in the previous paper, c being expressed as the number of gramme molecules in 1 litre. For haemoglobin they are gramme equivalents, that the absorption coefficients might be directly comparable with those of the other pigments. The results obtained for opossum haemoglobin in the micrometer cell agreed with the results for this substance in the tonometer cell. The concentrations of substances in the haemochromogen experiments were so chosen that dissociation was negligible.

DETAILS CONCERNING SOLUTIONS.

For Reduced Haemoglobin using tonometer tube 0·956 cm. in depth:

1. Opossum Haemoglobin 0·494 grm. per litre. To photograph head of band 0·0494 grm. per litre.

2. Rabbit Haemoglobin 0·231 grm. per litre. To photograph head of band 0·115 grm. per litre.

For Reduced Haemoglobin using micrometer cell:

10% $Na_2S_2O_4$ in 5% NH_4OH. Cell depth 0·05 cm. Concentration of Haemoglobin = 0·654%. For head of band, same solution used with cell depth 0·01 cm.

For Reduced Haematin, using micrometer cell, the following three solutions were made up and mixed immediately prior to filling cell for photography:

1. Haematin solution 4·49 m. grms. Haemin in 5·0 c.c. 0·01 N. NaOH.

2. Gelatin solution 0·5% in 0·01 N. NaOH.

3. Reducing solution 0·043 grm. $Na_2S_2O_4$ in 5 c.c. 0·2 N. NaOH.

1·0 c.c. of No. 1, 2 c.c. of No. 2, 2 c.c. of 0·01 N. NaOH mixed, 0·5 c.c. of the reducer being added just before making up for photography.

For Cyanhaemochromogen as for reduced Haematin, but solution 1 contained 2·66 mgs. Haemin in 2·5 c.c., and solution 3 contained in addition 0·080 grm. KCN. Cell depths used = 0·1 cm. and 0·05 cm.

For Denatured Ox Globin Haemochromogen as above: Solution 1, 4·02 mgs. Haemin in 5 c.c. 0·01 N. NaOH; solution 2, 0·01999 grm. den. ox globin in 4·0 c.c. 0·05 N. NaOH; solution 3, 0·055 grm. $Na_2S_2O_4$ in 5 c.c. 0·2 N. NaOH.

1 c.c. of 1, 2 c.c. of 2, 0·05 c.c. of 3, mixed as previously explained. Depth of cell = 0·05 cm.

DISCUSSION.

A comparison of the absorption spectra of the various iron-containing derivatives of haemoglobin shows that these pigments group themselves in an interesting manner.

The intense band in the region of 4,000 Å.U. is shown by the four native pigments, haemoglobin, methaemoglobin, oxy- and carbon monoxy-haemoglobin, and, most strongly of all, by haemin in an organic solvent. It is shown as sharply, though with a less intense maximum absorption, by the two haemochromogens. Alkaline methaemoglobin, though a native protein, does not show this band, but has instead a modified band like that of alkaline haematin in alcohol. Acid and alkaline haematin in water and reduced haematin have very similar absorption spectra, consisting each of a broad, ill-defined band with a maximum at 3,800–3,900 Å.U., of small intensity compared with the main band of the other pigments, and, possibly, a very feeble band in the neighbourhood of 2,500 Å.U. According to Friedli (1924) the absorption coefficient of the main band of alkaline haematin in water is greater than we have observed. It is possible that he worked under special conditions which presented any agglomeration of the pigment. The degree of dispersion of haematin in alkaline solution is apparently altered by the amount of alkali present; strong caustic soda, for instance, precipitates sodium haematinate from its solution.

With regard to the subsidiary bands of the other proteins, haemoglobin

and methaemoglobin have one each; that of haemoglobin is extremely faint, while that of methaemoglobin is better defined. The addition of a gaseous molecule to the pigment results in the presence of two well-defined bands at 3,400 Å.U. and 2,500 Å.U., the exact positions differing for the two gases. The haemochromogens show the two subsidiary bands similar to the oxy- and carbon monoxy-haemoglobins. If the pairs of subsidiary bands are to be attributed to a common cause, it would appear to be in the saturating of the co-valencies of the iron atom by oxygen, carbon-monoxide, the cyanide group, and the tertiary nitrogen atoms in denatured globin respectively.

The most reasonable hypothesis would appear to be that the intense main band is present only when the pigment molecules are widely separated or the valencies of the iron atom fully utilized. This would cover the cases of haemoglobin, oxyhaemoglobin, carbon monoxyhaemoglobin, methaemoglobin, haemochromogen, cyanhaemochromogen, and of haemin in true solution in an organic solvent. In the cases of acid haematin in water and of reduced haematin the gelatin is probably insufficient to prevent a certain amount of agglomeration of the pigment. Alkaline methaemoglobin and alkaline haematin in alcohol have spectra somewhat similar to one another, and intermediate in type between those discussed above, as regards their main bands. The possibility is by no means yet excluded that these represent dynamic equilibria between the forms methaemoglobin-alkaline haematin protected by globin, and of acid haemin in alcohol-alkaline haematin in water, respectively. The alcoholic solution of alkaline haematin used contained about 10% water. These hypotheses at present lack experimental basis, but it is hoped to investigate them at a future date.

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TABLE 1.

Substance.	Head.			Foot.				Table.
	λ A°U.	$\nu 10^{-12}$	$\epsilon 10^{-3}$	λ A°U.	$\nu 10^{-12}$	$\epsilon 10^{-3}$	$\epsilon H/\epsilon F$	
Rabbit Haemoglobin	4,228	709	123	3,054	982	24·4	5·0	2
	2,720	1,103	37·0	2,565	1,170	34·0	1·08	
Opossum Haemoglobin	4,195	715	127	3,105	966	17·6	7·2	3
	2,627	1,142	28·2	2,500	1,200	26·2	1·1	
Denatured ox globin Haemochromogen	4,237	708	110	3,744	801	16·9	6·5	4
	3,323	903	31·4	2,940	1,021	21·7	1·4	
	2,705	1,109	27·7	2,607	1,151	26·5	1·05	
Cyanhaemochromogen	4,350	692	91·0	3,840	784	14·8	6·1	5
	3,380	889	24·7	2,950	1,018	16·5	1·5	
	2,640	1,138	24·7					
Reduced Haematin	3,935	762	44·1	3,100	968	12·9	3·4	6
	2,646	1,134	14·7	2,500	1,200	12·9	1·1	

TABLE 2.

Rabbit Haemoglobin.

$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.	$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.	$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.
655	9·7	745	59·1	1,089	37·0
659	11·8	748	51·8	1,117	37·0
670	34·0	818	39·2	1,170	34·0
679	73·9	844	37·0	1,205	37·0
686	108	900	31·8	1,229	39·2
694	118	945	26·6	1,240	41·3
709	123	982	24·4	1,255	44·4
726	118	1,019	26·6	1,271	59·1
735	108	1,055	31·8	1,279	73·9
742	73·9				

TABLE 3.

Opossum Haemoglobin.

$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.	$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.	$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.
655	8·8	789	32·5	1,125	28·2
662	10·5	814	31·5	1,160	28·2
665	19·3	861	28·2	1,190	26·5
674	35·2	878	26·4	1,200	25·5
679	51·9	897	22·9	1,212	26·5
694	105	917	21·1	1,235	29·9
701	116	948	17·6	1,255	35·2
709	127	985	17·6	1,271	51·9
722	127	1,009	21·1	1,288	91·5
726	116	1,044	24·7	1,304	116
735	105	1,070	26·4	1,327	140
742	51·9				

TABLE 4.

Denatured Ox Globin Haemochromogen.

$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.	$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.	$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.
656	7·2	718	79·1	876	28·9
666	9·6	720	71·2	903	31·4
670	12·1	726	57·6	937	28·9
674	16·9	733	43·4	973	24·1
682	38·6	739	38·6	1,007	21·7
686	45·8	745	33·8	1,033	21·7
690	57·6	764	24·1	1,062	26·5
694	79·1	778	19·3	1,109	27·7
701	106	801	16·9	1,151	26·5
705	108	812	19·3	1,181	28·9
708	110	822	21·7	1,232	33·8
709	108	844	24·1	1,299	41·0
710	106	863	26·5		

TABLE 5.

Cyanhaemochromogen.

$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.	$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.	$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.
638	3·3	718	48·5	989	18·0
645	4·9	726	32·9	1,005	16·5
655	9·8	735	26·3	1,031	16·5
662	14·8	757	18·0	1,047	18·0
670	32·9	766	16·5	1,073	21·4
676	55·9	784	14·8	1,117	24·7
678	59·3	801	16·5	1,160	24·7
684	91·0	848	23·0	1,207	23·0
701	91·0	889	24·7	1,250	19·7
709	72·1	922	23·0	1,293	16·5
713	59·2	967	21·4	1,327	11·5
715	55·9				

TABLE 6.

Reduced Haematin.

$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.	$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.	$\nu 10^{-12}$ corr.	$\epsilon 10^{-3}$ corr.
635	7·3	731	36·8	917	14·7
648	9·3	762	44·1	937	12·9
655	11·0	794	36·8	1,002	12·9
662	12·9	820	29·4	1,134	14·7
670	16·5	873	20·2	1,200	12·9
679	20·2	908	16·5	1,288	14·7
709	29·4				

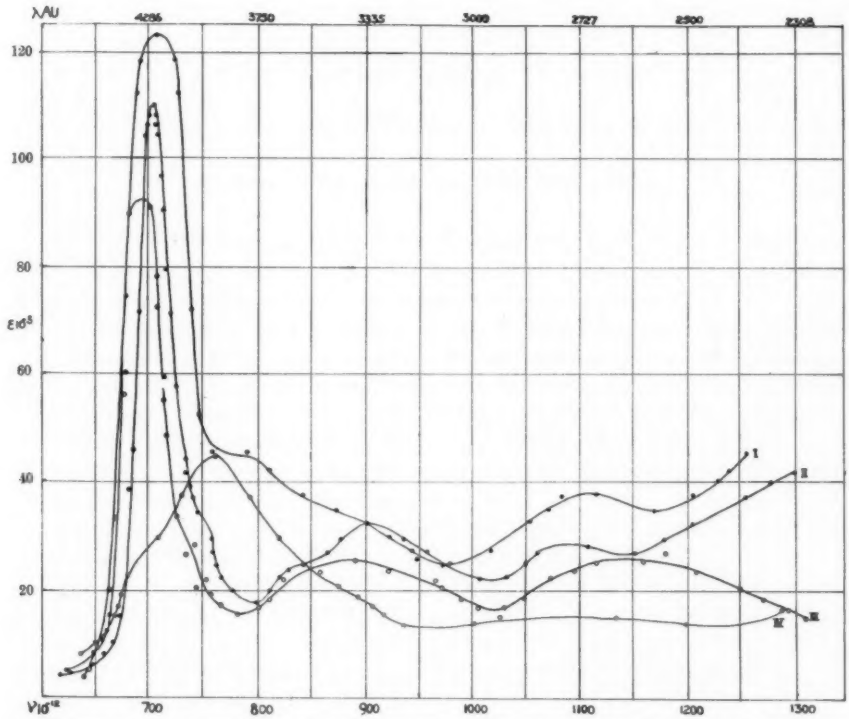

Figure. 1. I Rabbit Haemoglobin. II Denatured Ox Globin Haemochromogen. III Cyanhaemochromogen. IV Reduced Haematin.

STUDIES IN CENTRE OF GRAVITY CHANGES

No. 4

ON THE MEASUREMENT OF THE "BLOOD SHIFT" TO THE ACTIVE MUSCLES IN STAIRCASE RUNNING BY MEANS OF THE C. OF G. BALANCE TABLE.

by

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In a previous communication (this Journal, vol. 8, p. 53) a method was described for recording graphically changes in the centre of gravity of the body by means of a recording balance table supporting the subject.

It was found that when a subject, after exercising the lower limbs by running up and downstairs, lay recumbent on the balance table, there ensued a gradual movement of the centre of gravity towards the head. This was interpreted as mainly due to the return of the blood from the exercised muscles in re-establishment of the resting distribution. The term "blood shift" will be used as a convenient phrase to refer to this phenomenon, with the reservation that some additional factors may also be concerned.

This "blood shift" may be measured with considerable accuracy in terms of the change in the supporting force at the end of the balance table.

The sensitivity of the apparatus is indicated by the fact that the placing of a 20-grm. weight at the end of the balance table will deflect the index about 7 mm. When the system (without the subject) is repeatedly displaced and allowed to reassume equilibrium, the mean error of return to original level is well under one-fifth of 1 mm.

When, however, the subject is lying on the balance table the mean position of the writing point cannot be determined with such precision, owing to the excursions produced by breathing. None the less, by suitable damping of these oscillations it is possible to determine this mean level with more than sufficient accuracy for the purpose in hand.

Damping was effected by attaching to the caudal end of the balance table a disk immersed in thick oil. The lag in changing level thus introduced was carefully investigated, and shown to involve no appreciable error.

The method adopted is to take a primary tracing with the subject on the table until no further change of level occurs, and then, after the subject has run two cycles of the staircase, to record a further tracing until it runs horizontally a second time.

Assuming that the subject has reached the same condition of rest each time the tracing runs horizontally, the change of mean level of the post-exercise tracing affords a measure of the "blood shift." By measuring the mean total displacement of the writing point as so much length of arc, and calibrating against a known weight hung on the hook of the *balance alone*, we may express the apparent displacement of centre of gravity in quantitative terms, e.g., suppose the arc swept out by the balance index from immediate post-exercise level to final level is the equivalent of hanging X grms. on the balance alone, then the apparent displacement of mass in the subject is the equivalent of transferring X grms. from the fulcrum to end of the table (where it is supported by the spring). Since this distance is, in the case of balance table No. 1, 120 cm., the apparent "blood shift" is assessed as 120 X grm. cm.

It is found that when a subject reclines upon the balance table for the preliminary period the cranial end of the system sinks steadily for some 20 minutes or so, indicating a progressive migration of body fluid from the lower limbs. The interpretation of these changes will be discussed later in relation to certain experimental work carried out by Waterfield on the fluctuation of limb volume due to changes in posture. It is sufficient to note here that even after the standing and slight activity involved in ordinary laboratory work, a moderate period of time is necessary to attain a constant level of tracing. The effect of exercise should therefore be discriminated from the effect of the upright posture during the exercise. To this end an additional tracing was taken in the case of each subject to determine the effect of standing at rest for a period some minutes longer than the duration of the exercise. The sequence of the experimental periods for each subject was therefore as follows:

1. The subject comes from ordinary laboratory activity and reclines upon the balance table until constant level is attained. In general a moderate change of level is recorded over a period of some 20 to 25 minutes.

2. The subject stands up for 5 minutes, and thereafter a second record is taken. In general the change of level is very small, final equilibrium being reached in about 10 to 15 minutes.

3. The subject then runs twice up and down a flight of 62 steps (total height 36 feet) in about 1 minute and 20 seconds. The time is kept fairly constant by striking a bell each 5 seconds to correspond with the natural divisions of the staircase, so permitting the runner to maintain a schedule. The subject then continues the same pace from the staircase to the apparatus, climbs on to the balance table with a minimum loss of time, and adjusts himself quickly to a comfortable position. The drum, in general, is started with a delay of some 10 to 15 seconds.

Mosso (1884) also experimented with a balance table, but from a different

point of view. He sought to extend his plethysmographic work on limb volumes by this means, claiming to demonstrate a very considerable alteration of these volumes during respiration. Subsequent critics have pointed out, however, that the major change that he registered in this way was really due to the displacement of the viscera by the diaphragm. This criticism finds confirmation in a previous communication of this series, showing that great variations in tracings are produced by voluntary movements of the diaphragm. Mosso's balance table, moreover, was not readily adaptable for quantitative study, since it was constructed with a huge keel to maintain it in stable equilibrium with the subject in position. This would therefore require still more elaborate calibration than the present system, which is provided with at least one self-registering quantitative unit (the spring balance). Mosso recorded that after a subject first reclined upon the balance there ensued a steady migration of body fluid from the lower limbs, which persisted for some time. He roughly evaluated this by placing a vessel near the feet, and adding water to it at intervals to keep the balance table level, and he assumed that the total amount of blood which left this region was equal to the volume of water added.

Weber (1907) employed Mosso's balance to investigate alterations of blood distribution produced by psychological causes, and in the few quantitative measurements followed the doubtful technique employed by Mosso.

A typical tracing, taken after the staircase running, is shown in fig. 1, together with another tracing discussed subsequently.

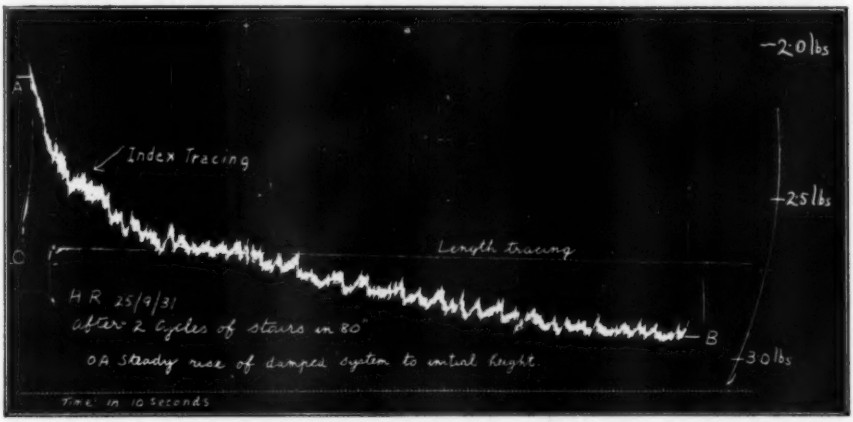

Figure 1.

Showing the progressive fall of the index tracing after the staircase run. The calibration is shown at the side in pounds, being marked directly from the readings of the index on the face of the spring balance.

The initial rise OA is produced by making a small movement of the sliding weight attached to the balance table so as to make the cranial end a little lighter. This is done immediately after the writing lever is applied to the drum surface, which in turn is done as soon as the initial equilibrium is established.

This procedure involves a loss of not more than about 10 seconds, as indicated by previous graphic investigation of the curves of lag. At the same time it ensures that no downward movement of delayed equilibrium produced by lag can possibly complicate the true initial fall due to the "blood shift," which is frequently quite as rapid as shown in this tracing.

The actual extent of the change may best be gauged by reference to the distance AO, which is equal to 8·7 cm.

INVESTIGATION OF VARIOUS FACTORS OTHER THAN "BLOOD SHIFT" WHICH MAY INCREASE THE POST-EXERCISE DISPLACEMENT OF CENTRE OF GRAVITY.

These possible factors are: 1. An exaggeration of the tilting effect due to the fact that this very change carries the centre of gravity of the whole system a little farther from the fulcrum, thus setting up an increased moment. 2. Any alteration in the mid-position of the diaphragm in the change from enhanced to normal breathing. 3. Any alteration in the length of the subject during the record period.

1. This change has been discussed in a previous communication (this Journal, vol. 10, p. 97), where a method devised to measure the height of the centre of gravity of the whole system above the fulcrum was described.

Calculations[1] made from the resulting data show that this exaggeration amounts to 7 per cent. of the gross change in the case of one balance system employed, and to 3 per cent. in the case of another, and that variations in body weight do not introduce errors greater than about 1 per cent. A mean body weight was therefore assumed for all subjects, and the above corrections applied to the original data.

2. Since the damping of the system produced a lag in the response to the respiratory changes of centre of gravity, the corresponding excursions of the tracing depart little from the mean level, and no abrupt change can take place. It follows, therefore, that any significant alteration of mean position of the diaphragmatic excursions prevailing for a period well beyond the lag period would produce a distinct change in level of the tracing. Hence if this mean position of the diaphragm was appreciably more caudal during the enhanced

[1]The correction factors concerned under different circumstances were calculated, since direct calibration (under static conditions) by placing a known weight on the end of the balance table was not feasible, owing to the respiratory variations of level; nor can these be eliminated by holding the breath, since this very procedure leads to a progressive change of level.

period of breathing immediately after exercise, then during the period of quiet breathing at the end of the tracing this mid-diaphragmatic change might be responsible for a considerable part of the total change of level observed.

The most apposite data in this respect are the measurements of diaphragmatic movement by means of the orthodiagraph, recorded by Halls Dally (1908). He publishes observations on 15 healthy subjects of each sex, for both right and left portions of the diaphragm, recording in each instance the position at the four cardinal phases of respiration, namely, normal inspiration and expiration and maximal inspiration and expiration.

Contrary to expectation Dally's data show that the mean diaphragmatic position is displaced in the *cranial* direction when normal breathing is superseded by *maximal* respiratory movements. This was found to be the case in 26 out of the 30 observations on the men and in 25 out of 30 observations on the women.

Although this does not prove that the *enhanced* breathing of the immediate post-exercise period causes a mean diaphragmatic displacement in the same direction, it renders highly improbable the occurrence of a reverse change under these circumstances of sufficient magnitude to invalidate our interpretation of the phenomenon in question as chiefly a "blood shift."

The experimental investigation of this question was carried out as follows:

The oil damper was first removed, so that the system could respond to any altered distribution of mass within a fraction of a second. Next the subject,[2] while lying supine upon the balance table, was required to breathe deeply for about 30 seconds, in order to prepare himself for holding the breath. He was then required to cease breathing, except when and how directed. In this way a series of tracings could be inscribed upon the moving drum corresponding to normal inspiration and normal expiration, by keeping the subject at each of these respiratory phases for some 3 to 5 seconds. The mean of these levels would then correspond to the mean position of the diaphragm under conditions of normal breathing. Next, while the subject is still breathing or not, as directed, he is required to vary in the same way between deep inspiration and deep expiration, an attempt being made to reproduce as far as possible the extent of such deep breathing as would be brought about by the staircase run. Here again a series of up and down levels was traced out, so that the mean level of all would correspond to the mean position of the diaphragm under the conditions of enhanced breathing. Lastly, before reverting to normal breathing, a series of tracings similar to the first was obtained, in order to eliminate any progressive change that might be taking place in mass shift. Finally the mean levels of the first and second series of tracings were compared with the mean level of the middle series. These observations were made on five subjects, all of whom showed higher mean levels with deeper than with normal respiration. The difference between these final levels then indicates the extent to which the factor in question enters into the gross extent of the apparent "blood shift" as measured. The results are set out in Table 1.

[2]Only experienced subjects were used for this experiment.

TABLE 1.

Showing the Increase of Level of the Tracing taken as described, due to the Caudal Displacement of the Mean Position of the Diaphragm from the Normal Mean Position as a Result of Deep Breathing.

No. of Subject.	Increase of level of tracing in cm.
1	0·6
2	0·5
3	0·9
4	0.6
5	1·1
Mean	0·74

These results, although of no great magnitude, appear at first sight to conflict with the conclusions drawn from the published data of Halls Dally. It is quite possible, however, that in most subjects, when maximum inspiration is made, the thoracic movement is involved to such an extent as to flatten the abdominal muscles, so preventing the descent of the diaphragm to such an extent as could take place in moderately deep breathing. If this were so it would account for the difference of Halls Dally's data from our own.

The mean change of 0·74 cm. corresponds to about 7 per cent. of the mean total change of level, so that it would appear from this that we have to deal, not with a major factor, but merely one that is sufficiently large to be corrected for. Since these effects are much more variable than the weights of the subjects, and are, moreover, rather small, they have not been expressed in terms of change per unit of weight, but a uniform deduction of 7 per cent. was made from all the primary measurements.

3. Before discussing the influence of changes in length, it is important to describe how precautions were taken to keep the subject in a constant position throughout the experiment.

Upon climbing on to the balance table the subject assumes the supine posture, with the knees just slightly flexed, and soles of the feet pressed against a foot-plate at the end of the table. He then straightens out so as to push the body just perceptibly farther along the table, and so makes still firmer contact with the foot-plate. This firm contact is maintained throughout the experiment, and therefore serves to standardize the position of the subject. Suitable elbow-rests are provided to make the subject comfortable.

It is well known that the height may vary from time to time under different circumstances; e.g., a person is invariably shorter in the evening than in the morning, and recovers his height after a night's sleep. Other observers again record a decrease in height after carrying heavy loads, and a salient example is quoted of a marked shortening in the case of a subject after all-night dancing.

It was not expected, however, that one to three minutes' exercise would produce much change in length, but it was thought advisable to take sufficient precautions to eliminate it from consideration.

The following experiments were therefore planned to measure any such change with

great accuracy by means of a cathetometer placed horizontally and parallel to the long axis of the balance table. To this end a small piece of paper marked by a dot (to serve as a sight for the telescope of the cathetometer) was gummed to the lobe of the subject's ear. Immediately upon the assumption of the supine posture by the subject (who had just run four cycles of the staircase in 3 minutes 3 seconds), a reading was taken, and thereafter a series of such in fairly rapid succession. The experiment revealed a surprising amount of change in a relatively short time, namely, a decrease of about 5·4 mm. in some five minutes. Since the soles and heels of the subject maintained firm contact with the foot-plate throughout the experiment, these altered readings must indicate a shortening in total length[3] of the subject. In this experiment only a few readings were taken on the down trend of the curve, so that the whole procedure was repeated with a view to obtaining as many readings as possible in

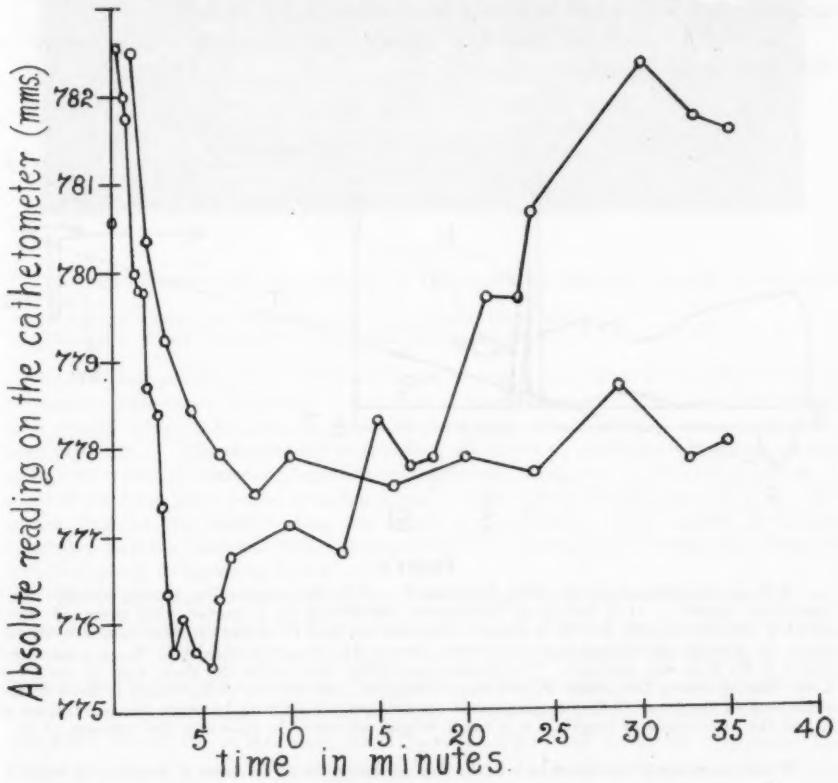

Figure 2.

Showing two types of change in length of the subject when supine during the post-exercise period. (Measured by the cathetometer.)

[3]Subsequent experiments show that this change is not due to head movements, so that a real alteration of length does take place.

the early stage of rapid change. Essentially the same effect was obtained. The data from this experiment, and also for a subsequent experiment showing a different type of result, are plotted as curves in fig. 2.

The direction in the change in length is here significant, for in these two instances a shortening occurred. This shortening must oppose the effect of the "blood shift" which proceeds in a cranial direction, and since in both these cases quite a substantial "blood shift" was recorded, it is clear that the alteration in length cannot exert any relatively great influence on the gross result.

Since, however, this factor may be large enough to be of relative importance, it was necessary to make some attempt to evaluate it, and to this end arrangements were made to record the changes in body length.

The device used for recording changes in the length of the subject is indicated in fig. 3 below.

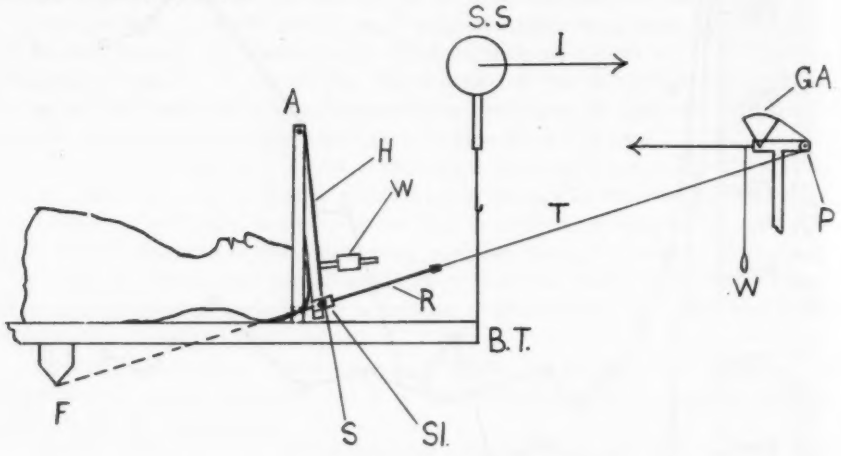

Figure 3.

B.T. is the balance table, with fulcrum F. S.S. the supporting spring balance with recording index I. H a swinging head-piece, consisting of a rectangular piece of wood pivoted about the axis A. W a weight attached to this to maintain firm pressure on the head. R a brass rod sliding in a cylindrical sleeve, SL, attached rigidly to H. s a small set screw to fix R in any position. T a thread connecting the rod to the short arm of the lever L by passing round the pulley P, and then along the grooved arc G.A., which is fixed to the short arm of the lever. (This arrangement ensures proportionality between the excursions of L and the alterations of length.) w a small weight necessary to maintain the tautness of T.

When the subject increases in length, H is advanced so as to cause a drop in the lever L. By calibration it was found that an increase of 1 cm. in length cause a swing of 5·6 cm. of arc of the lever L. It should be stressed that it is necessary for the rod R to be situated in the line FP. If this were not the case an appreciable error may be introduced, in that the mere tilting of the board would alter the length sP, and so produce an apparent change in length. This source of error is negligible with the rod in the line FP. The type of tracing obtained by means of this device is shown by tracing 2 of fig. 4, and also by the corresponding tracing of fig. 1.

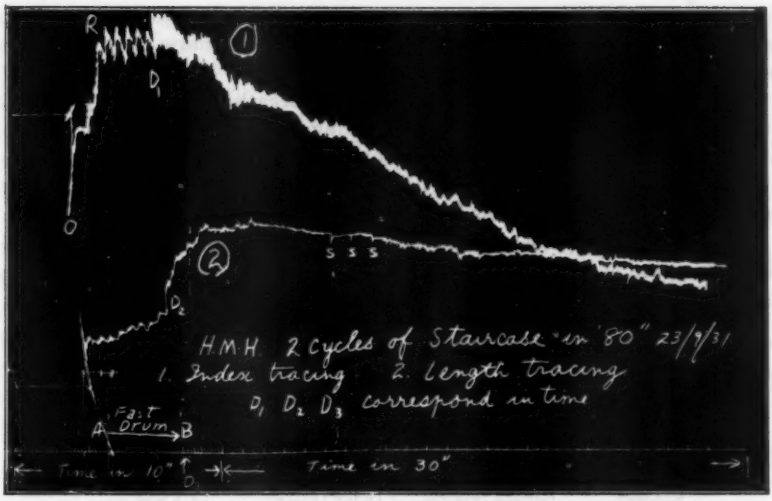

Figure 4.

Tracings obtained after the subject has performed the standard exercise. (Staircase running.)
Tracing 1. From the balance index (recording "blood shift").
Tracing 2. From the lever L (recording change of length).

In this instance the subject shortened to begin with, and subsequently regained some of his length. This shows a contrast to the corresponding tracing of fig. 1, where the subject grew steadily longer throughout the period of the record. The ascent OR corresponds to the ascent Oa of fig. 1. The rise of level of the index tracing at D_1 corresponds to the shortening at D_2. This sudden change of length is only apparent, being due to the abrupt change of speed of the drum produced by changing gears, as indicated by the time tracing at D_3. The letters S.S.S. on the length tracing are caused by swallowing, which is quite a common occurrence in these tracings. This has frequently been checked up by actual observation of the simultaneity of these two events.

The correction to be applied for this change in length of the subject was assessed by direct calibration with the subject in position on the balance table, the procedure depending on the fact that it is a simple matter for the subject to increase his length a little by voluntarily straightening out. It is necessary, however, to ensure that this lengthening is uncomplicated by any rotation of the head about the atlanto-occipital joints. This was done by requiring the subject to wear a pair of spectacles fitted with extensions from the side-pieces so as to project some 8 inches in front of him. The ends of these extensions were joined by a thread, which could be kept sighted in line with the reflection of the eyes in a horizontal mirror placed some 20 inches above the subject's head. In this way the head can be maintained in constant posture, even when voluntary changes of length are being made. In order to ensure a reasonably

constant level of the index tracing, corresponding to each change of length, the subject was required to hold the breath (after preliminary deep breathing) throughout the experiment.

When all was ready the subject held his breath, and then by direction straightened out and relaxed alternately, being kept in each position just long enough to establish level. By determining the difference between the mean levels of the upper series and of the lower series in the case of each tracing the required calibration was obtained.

The result was expressed as so much change in level of the index tracing per cm. alteration of the length tracing. The results of a series of such observations on four subjects are given in Table 3.

TABLE 3.

Showing the Calibration of the Length Tracing by Comparison with the Index Tracing.

Subject No.	Difference in level (cm.). Length tracing B.	Difference in level (cm.). Index tracing A.	Change of level of A per cm., Change of B.
1	2·3	1·5	0·65
2	4·8	3·9	0·81
3	5·1	2·2	0·43
4	1·9	1·1	0·58
Mean	—	—	0·62

In some experiments the change between initial and final levels of tracing B during the post-exercise period amounted to as much as 20 to 30 mm. The average change, however, was only 7 mm., equalling 4 mm. of arc of tracing A. Since the mean total drop of the index amounted to 97 mm. of arc, this is equivalent to about 4 per cent. of the gross change.

When the individual changes of length are examined, however, we find very variable effects, some subjects showing a shortening and others a lengthening during the post-exercise period. Of the 74 subjects observed about 80 per cent. increased in length in this period, while the remainder shortened.

We may now illustrate by an actual example how the raw data were treated.

In the case of subject No. 59 (weight 67 kgs.) the depression of index arc amounted to 74 mm., while the length tracing showed a drop of 14 mm.

Since by Table 3 14 mm. drop[4] in tracing B = 0·62 × 14 = 8·7 mm. drop in tracing A, this reduces the gross change to 74 — 8·7 = 65·3 mm. of arc.

Next a deduction of 3 per cent. must be made to correct for the exaggeration of effect due to factor 1, and a further deduction of 7 per cent. to compensate for the change of mid-position of the diaphragm, making 10 per cent. in all. This reduces the value in question to a net 58·8 mm.

Now in the case of this balance, 1 mm. of arc = 6·01 grms. So that our net value

[4]These measurements were not made in terms of arc, since they were so small as to closely approximate the vertical drop.

= 58·8 × 6·01 = 353 grms., approximately, and since the spring balance is 120 cm. from the fulcrum, the displacement of mass concerned = 353 × 120 grm. cm. = 42,360 grm. cm. Therefore, the net "blood shift" in this case per kg. of body weight = 42,360/67 grm. cm. = 630 grm. cm. approximately.

The observation of mass displacement for the three experimental periods in the case of each subject were considered in this way, and the results expressed to the nearest 10 grm. cm. The essential results are given in the next section.

PRESENTATION OF THE DATA.

The data here concerned fall naturally into two divisions: A set of observations made on a series of subjects; a series of observations on the same subject.

The subjects upon whom the following observations were made were all male medical students (56 in number), mostly from nineteen to twenty-two years of age. The exercise consisted in running twice up and down a 36-feet flight of stairs in about 80 seconds. the rate of running being controlled by the striking of a bell. From this we may calculate a minimum value for the mean rate of working, using Benedict's (1928) estimate that the work done in running downstairs is about one-third of that done in running upstairs.

On this basis the total work done is equivalent to climbing $\frac{4}{3}(2 \times 36) = 96$ in 80 seconds. Since the average weight of the subjects amounted to 143 lb. this is the equivalent of 96 × 143, that is, approximately 13,700 foot lb. in 80 seconds. The mean rate of working is therefore about 10,300 foot lb., or 730 kg. metres per minute, which is nearly one-third of a horsepower. There was a considerable amount of variation in the extent of the "blood shift" in different subjects, not only after the exercise, but also after ordinary laboratory activity, and after the 5 minutes' standing period subsequent to some 30 minutes' recumbency. The statistical values for the data obtained after the three experimental procedures are given in Table A. For convenience of reference the three experimental periods are termed A, B, and C in the actual time sequence, namely, A after ordinary laboratory activity, B after standing for five minutes at the end of period A, and C after the staircase run which immediately followed period B.

TABLE 4.

Essential Data concerning the "Blood Shift" during the Three Experimental Periods.

	"Blood Shift" in gm. cm. per kg. of body weight after:		
	Ordinary Laboratory Activity.	After 5 minutes' Standing.	After Staircase Running.
Mean	255	136	471
P.E. of Mean	9·4	8·3	12·3
S.D.	98	88	128
Coefficient of Variability	40·6%	64·8	27·2%
Lower Quartile	170	80	391
Median	233	122	436
Upper Quartile	325	200	539
P.E.	77	60	74

The data show that the "blood shift" after ordinary laboratory activity is of the order of twice the change after the period of five minutes' standing, while that after the exercise is about twice as much again. The change recorded after the five minutes' standing is the most variable, while that after the exercise is the least variable.

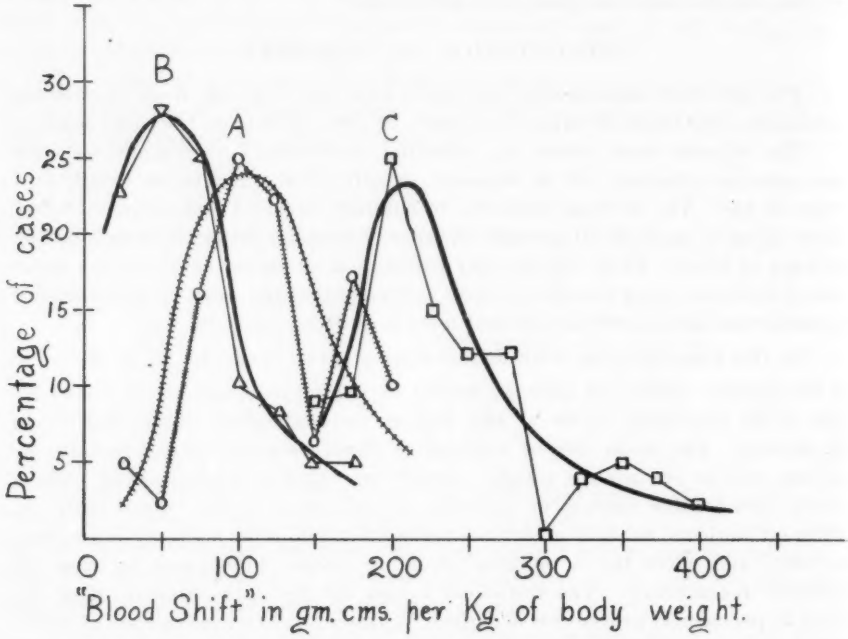

Figure 5.

Frequency curves for: A, the "blood shift" after ordinary laboratory activity. B, the "blood shift" after a period of 5 minutes' standing. C, the "blood shift" after the staircase run.

So far the results have been expressed as a "blood shift" of so many gm. cms. per kg. of body weight, this being necessary in order to consider all the individual data as one series, since the change is clearly a function of the body weight.[5] In order to give some idea as to the actual extents of mass shift in the

[5]The change is in all probability also affected by height, over and above the allowance made for weight, since in the case of two subjects of the same weight, and with the same mass concerned in the "shift," the distance factor would be greater in the taller of the two. Since, however, the actual results as expressed in gm. cm. per kg. of body weight showed a far greater variability than the heights of the subjects, the writer forbore to adopt the unnecessarily complex concept of so many gm. cm. of mass shift per kg. metre of subject.

He is well aware, however, that in refraining from so doing the correlations given a little later are thereby somewhat increased. The extent of this increase is probably rather small, and would not alter the fact that the definite relationships indicated do really exist.

three circumstances we may compute the mean values concerned. Since the average weight of the subjects was about 65 kg., the actual mean "blood shift" amounts to 16·6 kg. cms. after ordinary laboratory activity, 8·9 kg. cms. after five minutes' standing, and 30·6 kg. cms. after the staircase running. Since we do not know the distance of the shift we cannot at this stage form any estimate as to the additional amount of blood present in the working muscles concerned in contrast with that present at rest.

In order to give a comprehensive idea of the ranges of value for the three series, the frequency curves plotted in fig. 5 were drawn.

Of the three curves, B and C are decidedly of the skew type, in both instances the tendency to variation being distinctly marked in the upper values of the series, thereby lifting the average values well above the respective modes, and even about 10 per cent. above the respective medians, as seen in Table 4.

In studying the time relations of the "blood shift" a difficulty arises in fixing any definite point in time as the conclusion of the process.

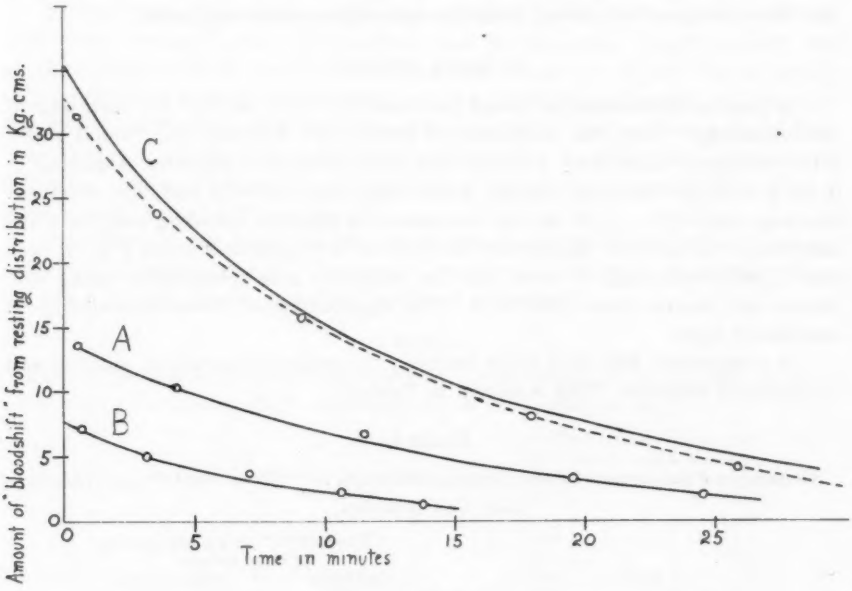

Figure 6.

Time relations of the "blood shift" in the three experimental periods, A, B, and C respectively.

An attempt was made, therefore, to average the results by determining in each case the various times for the completion of $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, and $\frac{7}{8}$ of the total

"blood shift," and to amalgamate these values to form a representative time curve. This was done by dividing the arc swept out by the index from initial to final level into the corresponding fractions, and then by drawing lines parallel to the time tracing to find the points of intersection with the mean trend of the tracing. By dropping vertical lines from these points on to the time tracing the times required were found. The curves obtained in this way are shown in fig. 6.

The times occupied by these changes increase with the extent of the "blood shift" concerned.

An error in the time relations is introduced in the tracing, in that the sweeping out of an arc by the descending index must change the relative vertical alignment.

This has been corrected for in the tracing C of fig. 8, where the time relations as recorded are shown by the broken line and the correct time relations by the full line. This error is too small to be clearly shown in the case of the other two curves, where the amplitudes of arc swept out are much smaller, so that they are given as plotted from the actual measurements made.

CORRELATIONS.

A positive correlation is found between the "blood shifts" for each pair of circumstances. Thus the coefficient of correlation between the "blood shift" after ordinary laboratory activity and that after five minutes' standing is $0{\cdot}53 \pm 0{\cdot}07$, between the change after laboratory activity and that after the staircase run $0{\cdot}39 \pm 0{\cdot}08$, and in the cases of 5 minutes' standing and the staircase run, $0{\cdot}57 \pm 0{\cdot}07$. Moreover, the ratio of the coefficient to its P.E. in each case is sufficiently high to show that the odds of a true relationship being concerned are greater than 1,000 to 1. The significance of these relationships is considered later.

A comparison was then made between the responses given by athletic and non-athletic subjects. This is shown in Table 5.

TABLE 5.

Showing a Comparison of the Average Extents of "Blood Shift" in Athletes and Non-Athletes.

After:	"Blood Shift" as gm. cm. per kg. of body weight. Athletes.	Non-Athletes.
Ordinary laboratory activity ..	286	230
Standing for 5 minutes	168	109
Staircase running for 80 minutes	473	469

No significant difference appears after the staircase run, but distinct differences are shown in the effects of ordinary laboratory activity and of the five minutes' standing. The writer is not at present prepared to offer an explanation for these differences.

B. A SERIES OF OBSERVATIONS ON THE SAME SUBJECT UNDER DIFFERENT CONDITIONS.

Altogether some 30 odd systematic observations were made upon the same individual after some preliminary experience. Since he was thus regarded as a trained subject an attempt was made to eliminate the necessity for a correction for change in length by endeavouring to keep it constant throughout the experiment.

The method adopted was to place a mirror horizontally above the subject's head, and require him to wear the spectacles plus extensions previously described. Upon settling into a comfortable posture, he sighted the thread joining the spectacle extensions in the same line with the reflection of his eyes in the mirror. Next the absolute position of the eyes was fixed by moving a sliding piece attached to the mirror to bring a fixed mark on it into the same line of sight. By very little effort he was thus able to keep this fixed mark in the line of sight, and so maintain constant length during the experimental period. In these experiments the apparatus for recording changes in length was also employed as a control, and invariably traced a nearly horizontal line. The results were expressed directly as kg. cm. of "blood shift."

In the group investigation the object was to prove that a definite "blood shift" was brought about by exercise, and to determine approximately the average amount of the change. In the present series the object was to obtain sufficient data to give an approximate value to the different factors concerned in the "blood shift" under different circumstances. For this purpose observations were made: 1. After ordinary laboratory activity. 2. After the staircase run following ordinary laboratory activity. 3. After the staircase run following a 30-40 minute period of recumbency.

In each case not less than ten observations were made in order to obtain reasonably accurate means. The results are set out in Table 6, where the values are given in each case in ascending order of magnitude.

TABLE 6.

Various Determinations of "Blood Shift" under Different Conditions in the Case of H.R. Values given in Ascending Order.

	"Blood Shift" in kg. cm.		
	Effect of laboratory activity.	Effect of Staircase Run after ordinary laboratory activity.	Run after 30-40 minute recumbency.
	14·0	36·5	31·5
	17·0	39·0	32·0
	19·5	39·5	32·0
	19·5	39·5	33·0
	20·0	40·5	33·5
	21·0	41·0	34·0
	22·0	41·0	35·5
	22·0	41·5	36·5
	23·0	41·5	38·0
	24·5	41·5	39·5
	—	42·0	—
Mean	20·2	40·3	34·1
S.D.	3·11	1·50	2·55
Coefficient of Variability	15·4%	3·7%	7·5%

The most concordant set of results was obtained in the case of exercise following laboratory activity (coefficient of variability 3·7 per cent.), while the most variable were those after laboratory activity (coefficient of variability 15·4 per cent.). The several factors operating to produce these effects, and the attempt to evaluate them, are considered in the next section.

DISCUSSION OF THE RESULTS.

The extent of the "blood shift" after simple standing for 5 minutes shows clearly (*vide* Table 4) that the change of posture cannot account for more than about 30 per cent. of the amount of the "blood shift" after the exercise. Now the upright posture was maintained for not more than two minutes altogether in the case of the staircase run, including the time to get off and on the balance table. If we allow for this a postural effect even up to half the amount due to the 5 minutes' standing, this will only account for about 15 per cent. of the total change due to the exercise. Hence, unless the very process of capillary dilatation during the exercise leads to a considerable increase in the rate of filtration of lymph into the tissue spaces, the remaining 85 per cent. of the change is due to a shift of blood to the active muscles as a direct result of the exercise.

In the case of the "blood shift" observed on recumbency following ordinary laboratory activity, and also that after the five minutes' standing, two factors are probably concerned (affecting the lower portions of the body), inasmuch as there has occurred during the period of the erect posture: (1) A general increase in the total vascular volume of these lower parts; and (2) a passage of lymph from the capillaries into the tissue spaces of the same regions.

That the former change does occur is recognized by all previous work dealing with the effect of gravity upon the circulation.

The fact of increased accumulation of lymph in the lower limbs in the case of the erect posture is demonstrated conclusively by the experiments of Waterfield (1931), who shows that on standing after a period of recumbency the leg volume increases in two ways. Firstly, a relatively rapid and distinctly major change takes place, which he proves is due to increased blood volume; and secondly, a more gradual increase in volume occurs, extending over a period of about 40 minutes. Further, he offers substantial proof that this secondary change is produced by an accumulation of lymph in the tissue spaces.

In terms of the above it is easy to understand the correlation between the effects of simple standing and those of ordinary laboratory activity, which must be similarly affected by the erect posture. The correlation between each of these effects, however, and that of the staircase run, which occupies such a short period of time, is not so obvious.

In the case of the series of observations on H.R. there are sufficient data to evaluate the several factors concerned. This may be attempted in the following way:

Relatively to the recumbent state

Let P_1 be that fraction of the "mass shift" due to the gravity factor in causing the increased vascular volume of the prolonged erect posture.

P_2 be that fraction of the "mass shift" due to the accumulation of lymph in the tissue spaces during prolonged maintenance of the erect posture.

X_1 be that fraction of the "mass shift" due to the additional amount of blood mobilized in the muscles of the lower limbs during ordinary laboratory activity.

X_2 be the corresponding fraction of the "mass shift" due to the mobilization of blood in the muscles during the staircase run.

We may regard the "blood shift" observed after the staircase run following laboratory activity as involving the effects of exercise, the gravity effect on the vessels, and previous filtration of lymph into the tissue spaces. Hence from Table 6 we have:

$$P_1 + P_2 + X_2 = 40{\cdot}3 \text{ kg. cm.} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

Similarly the change observed after the staircase run following 30-40 minutes of recumbency may be regarded as involving the effect of exercise, and the gravity effect on the vessels, so that we have:

$$P_1 + X_2 = 34{\cdot}1 \text{ kg. cm.} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Finally the change after laboratory activity may be regarded as due to the sum of P_1, P_2, and X_1, so that we have:

$$P_1 + P_2 + X_1 = 20{\cdot}2 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Now in the previous discussion of the group results the relative values of P_1 and X_2 were assessed as 15 per cent. and 85 per cent. respectively of the total change concerned. Hence from equation (2):

$$P_1 = 5 \text{ kg. cm., and } X_2 = 29 \text{ kg. cm., approx.}$$

Substituting (3) from (1) we have:

$$X_2 - X_1 = 20 \text{ approx.}$$

Hence $X_1 = 9$ kg. cm. approx.

Substituting in (3)

$$5 + P_2 + 9 = 20 \text{ approx.}$$

Whence $P_2 = 6$ kg. cm.

Summarizing these results, our estimates of the mass shift from the resting recumbent state produced by the various factors are:

Produced by the gravity effect of vascular congestion—5 kg. cm.
Produced by accumulation of lymph in the tissues—6 kg. cm.
Produced by the exercise factor of laboratory activity—9 kg. cm.
Produced by the exercise factor of the staircase run—29 kg. cm.

It is of interest to note that the approximate equality of the first two factors is paralleled by Waterfield's estimates of these in producing an increase of leg volume on standing. He assesses this increase of leg volume due to lymph accumulation at about 50 c.c., and that due to vascular congestion at about 70 c.c.

APPROXIMATE LIMITING ESTIMATES OF THE SEPARATE MASS AND DISTANCE FACTORS IN THE "BLOOD SHIFT."

If one of the factors concerned in the "blood shift"—mass or distance—can be evaluated, the other is thereby fixed. Since, however, the absolute values

are not obtainable in either case, it seems worth while in the case of the staircase run to assess a minimal value for the quantity of blood concerned by determining a maximal value for the distance in question. This was attempted in the following way.

If we can fix a horizontal plane dividing the body approximately into a lower portion which includes the muscles concerned in the exercise, and an upper portion not concerned with the work, and if further we can assign a lower limit for the centre of gravity of the active muscles in the lower portion, and an upper limit for the centre of gravity of the whole upper portion, we may deduce that the "blood shift" proceeding after the exercise from the active to the inactive part, cannot be concerned with a greater mean displacement than the distance between these two limits.

The limit for the centre of gravity of the lower portion was determined by finding its centre of volume. (*Vide* appendix.) Let us call this point C. Now a brief consideration of the anatomy of this region is sufficient to convince us that the ratio of muscle to bone, and also to skin plus subcutaneous fat, is greater in the upper part of this portion than in the lower part. Hence the centre of volume of the non-muscular tissue will lie below the point C, while the centre of volume of the muscles concerned will lie above this point.

Although we may not be able to fix the height of the plane dividing the body into a lower active portion and a higher relatively inactive portion, we may readily assign limits between which it must fall. Thus a consideration of the distribution of muscular tissue concerned in the running shows clearly that this hypothetical plane must lie above the level of the greater trochanter and below the level of the anterior superior spine of the ilium. Again, in appendix 2 a method is described for determining an upper limit for the centre of gravity of the upper portion of the body in the case of the horizontal planes at each of these levels. The data obtained in this way are set out in Table 5.

TABLE 5.

Data obtained in regard to Centre of Gravity of the Active Muscles and of the Upper Relatively Inactive Part of the Body. Values are Percentages of Total Height.

Subject.	Height of the	Division of Body into Upper and Lower Portions by a Horizontal Plane at the Level of The Greater Trochanter.	The Anterior Superior Spine of the Ilium.	Index of Build.
	C.G. of the Active Muscles.	35·8	40·2	—
P.L.J.	C.G. of Upper Portion	70·5	74·0	1·03
	Difference	34·7	33·8	—
	C.G. of the Active Muscles	34·8	40·6	—
H.R.	C.G. of Upper Portion	70·5	74·0	0·97
	Difference	35·7	33·4	—

The subjects in this table were chosen as representing fairly diverse types of build, e.g., the index 1·03 signifies a relatively slim build, while the index 0·97 implies a relatively stocky build. More than 75 per cent. of the subjects previously measured showed an index within these limits. The relatively small degree of variation in these measurements is in keeping with the previous findings in regard to other measurements concerning the centre of gravity of the human body.

From this data a maximum value for the mean displacement of mass concerned in the "blood shift" may be reasonably assessed as 36 per cent. of the total height. This amounts on an average to $0{\cdot}36 \times 175 = 63$ cm. for all the subjects examined.

Since further the average "blood shift" due to the whole procedure amounted to 30·6 kg. cm., the minimum amount of mass concerned in the shift is $\frac{30{\cdot}6}{63}$ kg. = 485 grms. Again, since the period of exercise is so short, the total amount of lymph accumulation is probably small, so that *we may conclude in round figures that as a minimum estimate, rather less than 500 grms., or rather more than 1 lb. of blood, is mobilized within the active muscles, in the case of the staircase run, beyond the amount present in the resting recumbent position.*

SUMMARY.

1. A method for measuring the change of centre of gravity of the body during a period of recumbency following staircase running. The measurement is made by means of the centre of gravity balance table previously described. The result is expressed as so many kg. cm. of "mass shift."

2. Various factors are shown to contribute toward the apparent "mass shift" as graphically recorded.

3. These factors have been separately evaluated as follows:

(a) Effect of instrumental advancement of the centre of gravity due to the tilting of the balance table—about 7 per cent. of the gross apparent change with one apparatus and about 3 per cent. with another.

(b) Effect of change in mean position of the diaphragm due to the enhanced post-exercise breathing—about 7 per cent. of the apparent change.

(c) Effect of post-exercise alteration in the length of the subject—a variable amount corrected for by a special calibration.

4. The net change is referred to as the "blood shift," with the reservation that other minor factors are also concerned.

5. Data is presented summarizing the observations made on the "mass shift" relative to the resting recumbent state, due to:

(a) Ordinary laboratory activity,
(b) Five minutes' standing,
(c) Staircase running,

in the case of 56 young men.

6. A further series of observations was made on one subject under different conditions.

7. From the data so obtained an attempt was made to evaluate the separate effects of the various factors in altering the amount of body fluid in the lower portions of the body, namely:

(a) Changes in capillary volume due to the exercise.

(b) Changes in vascular volume due to gravity as a result of changes of posture.

(c) Changes of volume of the lower part of the body due to filtration or reabsorption of lymph.

8. By determining a maximum value for the mean distance concerned in the true "blood shift," an estimate is obtained of the minimum amount of additional blood mobilized in the active muscles beyond the resting quantity. The minimum value so arrived at for the average of all the subjects observed was of the order of 0·5 kg.

In conclusion, I wish to express my thanks to Professor H. Whitridge Davies, in whose laboratory the work was carried out, for his encouragement and criticism; to Associate Professor H. Priestley for a critical reading of the paper; and to Dr. A. J. Canny for checking the mathematical expressions employed.

APPENDIX 1.

The Determination of the Centre of Volume of that Portion of the Erect Subject Beneath a Given Horizontal Plane.

The method adopted for this purpose depends upon the principle that where the centres of volume of two known complementary fractions of a whole and also that of the whole are in question, the position of any of these points may be found by simple proportion from the positions of the other two.

To determine these quantities a uniform cylindrical tank was employed, some 42 cm. in diameter and 115 cm. high. By finding the volume of water required to fill it up to any given level, and again when the subject is standing in it, we may obtain the complete volume of the system and also the two complementary fractional volumes. Further, the centre of the volume of the whole is simply determined by taking half the total height, since primary calibration showed that the tank was remarkably uniform, e.g., when 15 separate volumes of water (10 litres each) were added in sequence, the increments of level showed a maximum individual variation of 1 per cent. Moreover, the first 7 litres increased the level by 47·60 cm., and the second 7 litres by 47·55 cm.

In order to find the centre of volume of the water present when the subject is standing in the tank, the level of water about the subject was increased in steps by adding 10 litres (measured out by weighing) at each step, and the increments of level were carefully measured by means of a manometer attached to the side of the tank. The final amount of water needed to reach the exact height in question was found by difference from the residue of the last 10 litre portion.

The volume of the subject to this level was found by reading the levels when the subject got in and out several times, care being taken when the subject got out to allow most of the water to drain back into the tank from the subject's body. Several such determinations agreed to less than 0·5 per cent.

Calculation of the Centre of Volume of the Water Surrounding the Subject to the Mean Level of the Greater Trochanters.[6]

Let H_O be the initial level of the water, namely, just touching the soles of the subject when standing on a flat board at the bottom of the tank; H_r the height of the water level when the rth addition of 10 litres is made; n be the total number of whole additions of 10 litres each; X the final amount of water added to reach the requisite level; H_t be the mean height of the greater trochanters; H_X be the height of the centre of volume of the final fraction X; then $H_X = \frac{1}{2}(H_n + H_t)$ approximately.

The height of the centre of volume of the $(r + 1)$th addition of 10 litres is equal to $\frac{1}{2}(H_r + H_{r+1})$. So that the products of each quantity added into the height of its centre of volume are respectively $\frac{1}{2}(H_O + H_1)$ 10, $\frac{1}{2}(H_1 + H_2)$ 10, $\frac{1}{2}(H_2 + H_3)$ 10, . . . $\frac{1}{2}(H_r + H_{r+1})$ 10, . . . XH_X. Therefore, since each of these quantities appears twice, except H_O, H_n, and H_X, the sum of all of these is equal to

$$10H - \tfrac{1}{2}(H_O + H_n) + X.H_X$$

and since the total volume of this added water = (10n + X) litres, the centre of volume of the whole mass of water

$$= 10H - \tfrac{1}{2}(H_O + H_n) + X.H_X/10n + X.$$

In the case of the subject P.L.J. this came to 48·32 cm. Further, half the height of the final level was 50·98 cm., the volume of water added was 112·4 litres, and the volume of the subject to the same level was 25·1 litres. Now it is a matter of elementary mechanics that if

S be the height of the centre of volume of that portion of the subject immersed,

W be the height of the centre of volume of the added water,

C be the height of the centre of volume of the whole (subject to level + added water), the ratio of the distances of S and W from C are in the inverse ratio of the volumes concerned.

Hence

$$\frac{S-C}{C-W} = \frac{112{\cdot}4}{25{\cdot}1} \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \quad (1)$$

Substituting the above values in this equation, we have

$$\frac{S - 50{\cdot}98}{50{\cdot}98 - 48{\cdot}32} = \frac{112{\cdot}4}{25{\cdot}1}$$

whence S = 62·9 cm.

In a repetition of the whole experiment (but taking the previous value for trochanter height), S = 63·1 cm., giving a mean value for S = 63·0 cm., which is 35·8 per cent. of the total height.

In a similar way two determinations were made of the height S in the case of the water level up to the mean heights of the anterior superior spines of the ilia. Here the duplicate determinations were 70·73 cm. and 70·95 cm., showing a highly satisfactory agreement. The mean value 70·8 cm. amounts to 40·2 per cent. of the total height of the subject, which is the value quoted for P.L.J. in Table 5.

[6]These levels were determined separately by repeated measurements of the midpoint between the upper and lower borders of this prominence as determined by palpation. It will be seen from the final results that even a considerable error in locating this level has a relatively small effect on the estimate of the distance factor in the "blood shift," since a compensatory error is thereby introduced into the location of the centre of gravity of the upper inactive portion of the body.

APPENDIX 2.

Determination of the Upper Limit of the C. of G. of that Portion of the Body above a Given Horizontal Plane.

This determination is based upon the fact that this point must be lower than the centre of the projection of this portion of the body upon any vertical plane. This follows from the fact that the lower portion of this part of the body has a greater average diameter than the upper part (which includes the head and neck), so that the discrepancy between the lower and upper masses must be greater, and therefore the effect in lowering the centre of gravity greater than in the case of the corresponding areas of projection. This must be so, since in the former case we are concerned with volume (a tridimensional quantity), and in the latter case with area only (a bidimensional quantity). The fact that the thorax contains so much air accentuates this discrepancy, so that the fixation of the upper limit for the centre of volume of the upper portion of the body in this way has an ample margin for certainty.

To find this centre of area a tracing was made of the outline of a man of average build (published by Bardeen), which was then transferred to a piece of cardboard, and the outline carefully cut out. This was then divided at a level corresponding to that of the greater trochanters, as determined on a percentage basis in relation to height, from the measurements of the present subjects. By the simple method of balancing this upper portion the height of the centre of area above the soles was found. This was 70·5 per cent. of the total height. In a similar way the height above the soles of the centre of the area cut off at the level of the anterior superior iliac spines was found to be 74·0 per cent. of the total height, which are the values quoted in Table 3.

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A RELATION BETWEEN SIZE OF HOST PUPARIA AND SEX RATIO OF *ALYSIA MANDUCATOR* PANTZER[1]

by

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Information on the ecology of *Alysia manducator* Pantzer has been published by Graham-Smith (1916, 1919), Altson (1920), Myers (1926, 1928), Holdaway and Evans (1930), and Morgan (1929). Additional observations have been made by Evans, and these will be published in the near future. The main facts which bear on the present dissecussion are as follows: The females parasitize larvae of various blowflies, oviposition taking place in larvae from half to full grown; in general a single egg is deposited in each host parasitized. Hatching of the parasite egg occurs just before or just after pupation of the host. Larvae of the host lie inert for a short time after being parasitized, and female parasites will not attack them in this condition. Altson considered that parasitized larvae after resuming activity are stimulated to burrow. Myers was not inclined to accept Altson's ideas. However, superparasitism is apparently prevented by these or other means, for Evans found that rarely were there more than one egg per host. When more than one egg is deposited in a larva only one parasite completes its development.

In the observations here recorded the hosts were *Lucilia sericata* Meig., *Calliphora erythrocephala* Meig., *Calliphora vomitoria* Linn., and species of *Sarcophaga*. At Toulouse the parasite is usually active in spring, early summer, and autumn.

The information contained in this paper was obtained from hosts parasitized under field conditions in the autumn, 1929. Baits were exposed on October 12, and within a few hours flies were attracted and commenced ovipositing. The

[1] The observations here recorded were made by one of us (F.G.H.) when he was engaged in a study of the biotic factors which play a part in regulating abundance of blowflies at Toulouse, in France. A brief summary of the main points which emerged from these studies has been given elsewhere. (Holdaway, 1930 b). The mathematical analysis of the data has been made by the other of us (H.F.S.). The information recorded in the present article was originally incorporated in the manuscript entitled, "Environment and Sex," referred to by Joyet-Lavergne (1931) in his book on the Physico-chemistry of sex.

first *Alysia* observed arrived at a bait on October 14, but the main period of parasitic activity was from October 15 to 18 inclusive. On October 18 cold weather set in, and put an end to activity of the adults.

Elsewhere (Holdaway and Evans (1939)) it has been shown that when *Alysia manducator* parasitizes *Lucilia sericata* in autumn it stimulates the host larvae to pupate when normally they do not pupate, but continue hibernating as larvae; a similar stimulation occurs also in *Calliphora vomitoria*. *C. erythrocephala* and *Sarcophaga* spp. apparently hibernate as puparia. Successfully parasitized hosts will therefore only be found among the puparia. The puparia removed from the soil should thus form a random sample of successfully parasitized hosts. Unemerged puparia were dissected for parasites. It was therefore possible to obtain a record of all parasites occurring in puparia successfully recovered from the soil. These are given in Table 1. Some of the puparia were damaged when being removed from the soil. As a result some of the parasites were prematurely killed, and their sex could not be determined. As will be seen from the table, the proportion of damaged parasites is very small, and in any case is probably a random sample.

The smallest puparia yielded male parasites, the largest female parasites, while a relation was found to exist between the sex ratio of the parasites emerging from each group of host puparia and the mean size of the pupara of the respective groups. The volume of a puparium, which is a prolate spheroid, is given by the expression

$$v = \tfrac{2}{3}\pi r^2 l$$

where r is half the minor axis and l is the major axis. Measurements were made by means of vernier callipers to a tenth of a millimetre. There is a slight error in the length measurements, due to the fact that the puparia were measured after the parasites had emerged. Emergence takes place through a hole made by the parasite in pushing off the anterior end of the puparium. The length measurements thus all fall short of the real length by a somewhat variable amount. Several puparia were measured before and after emergence of the parasites. From the measurements obtained it has been calculated that the standard error of the true volume estimated from measurements taken after emergence is $\pm 5{\cdot}1\%$.

The primary aim in obtaining the parasitism records was to determine the amount of parasitism in each species of blowfly. It was only after emergence of the parasites had been in progress some time that the possibility of a relation between sex and size of host suggested itself. Therefore no special measures had been taken for ensuring that the sex of the parasites emerging from the respective puparia should be known. In hosts other than *Lucilia* it was possible, in most cases, to link up to each puparium the sex of the parasite which emerged,

[2] In all the tables the volumes are given in terms of $\pi r^2 l$ or $\frac{3}{2}v$. The unit of volume is thus ·67 cub. mm.

but in the *Lucilia* batches about one-third of the parasites could not be associated with their respective puparia (*vide* Tables 2 and 6). Table 1 gives the total number of parasites, and their sexes, obtained from each host, together with the mean volume of the puparia yielding the parasites. Batches "1" and "2" are from separate baits exposed at the same time (October 12) under the same conditions. The puparia were removed from the soil 38 and 43 days later respectively. The data are represented graphically in fig. 1.

TABLE 1.

Showing the Relation between Size of Host and Sex Ratio of Parasites Emerging.

Batch.	Host.	Parasites. No. of Puparia yielding Parasites with Sex determinable.	Parasites. Males.	Parasites. Females.	Sex Ratio (Males as % of total).	Mean volume of Puparia in Column 3. Unit=·67 cub. mm.	Parasites emerged before measuring began. Males.	Parasites emerged before measuring began. Females.	Sex undeterminable.
1	*L. sericata*	124	98	26	79%	13·7	8	–	15
	C. erythrocephala	16	10	6	62%	28·7	1	2	–
	Sarcophaga spp.	12	3	9	25%	46·1	–	–	1
	C. vomitoria	9	2	7	22%	61·4	–	–	2
2	*L. sericata*	117	74	43	63%	19·4	–	–	8
	C. erythrocephala	15	6	9	40%	36·1	–	–	–
	Sarcophaga spp.	43	15	28	35%	43·1	–	–	–
	C. vomitoria	5	0	5	0%	69·2	–	–	–

Table 1 and fig. 1 suggest that the sex ratio of the parasite is related to the size of the host puparia. It is possible, however, that these data may represent a relation between sex ratio and the species of host. An attempt has therefore been made to determine the relation between sex ratio and size of host irrespective of the species of host, and also within each host species. Since it was not always possible to allocate to each puparium the sex of the parasite which emerged, the numbers available for studying these two relationships are smaller than those given in Table 1. Table 2 (columns 1, 6, and 7) and fig. 2 show the relationship when size of puparium is considered without reference to the species of host. This relationship agrees closely with that shown in fig. 1.

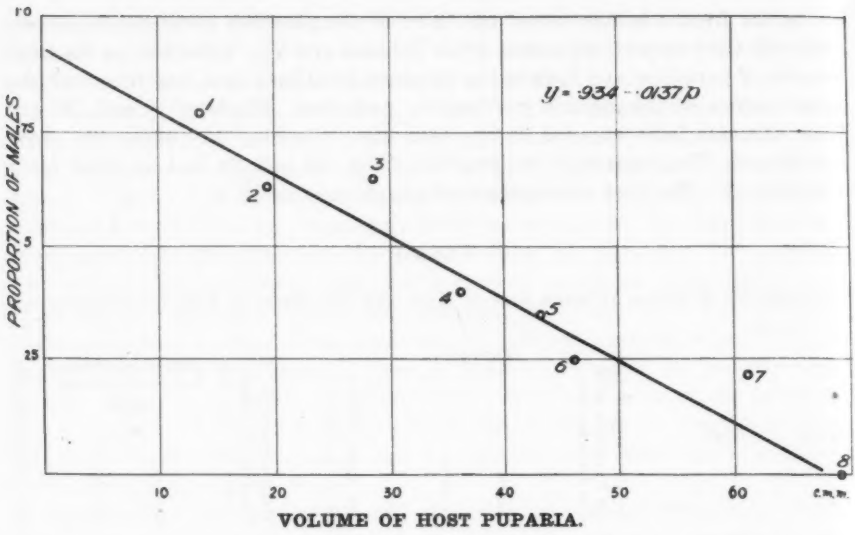

Figure 1. Regression line for sex ratio on size of puparia calculated from the means of samples of each host species. 1, 2 = *L. sericata* (124, 117). 3, 4 = *C. erythrocephala* (16, 15). 5, 6 = *Sarcophagids* (43, 12). 7, 8 = *C. vomitoria* (9, 5).

TABLE 2.

Number of Males and Females Produced by Each Sample of Puparia (only puparia with sex of parasite known used).

1	2		3		4		5		6	7
Host Batch	*L. sericata*		*C. erythrocephala*		*Sarcophaga spp.*		*C. vomitoria*		Total	Proportion of males.
	"1"	"2"	"1"	"2"	"1"	"2"	"1"	"2"		
Sex										
Size of Puparium										
8	34: 0	3: 0	—	—	—	—	—	—	37: 0	1·0
14	26:12	29: 6	0: 1	1: 1	1: 0	—	—	—	57:20	·74
20	4: 1	20:14	3: 0	1: 0	—	2: 1	—	—	30:16	·65
26	2: 1	5: 4	1: 0	0: 2	0: 1	3: 4	—	—	11:12	·48
32	—	2: 4	5: 3	2: 0	1: 1	2: 2	—	—	12:10	·54
38	—	0: 1	0: 1	1: 3	1: 1	3: 5	—	—	5:11	·31
44	—	—	—	—	0: 1	2: 3	—	—	2: 4	
50	—	—	—	0: 1	—	1: 3	1: 2	—	2: 6	
56	—	—	—	0: 1	0: 2	1: 3	0: 1	—	1: 7	
62	—	—	—	—	0: 2	0: 1	0: 1	0: 2	0: 6	·15
68	—	—	—	—	0: 1	0: 2	1: 1	0: 1	1: 5	
74	—	—	—	—	—	0: 2	0: 1	0: 1	0: 4	
80	—	—	—	—	—	0: 1	0: 1	0: 1	0: 3	
Total	66:14	59:29	9: 5	5: 8	3: 9	14:27	2: 7	0: 5	158:104	

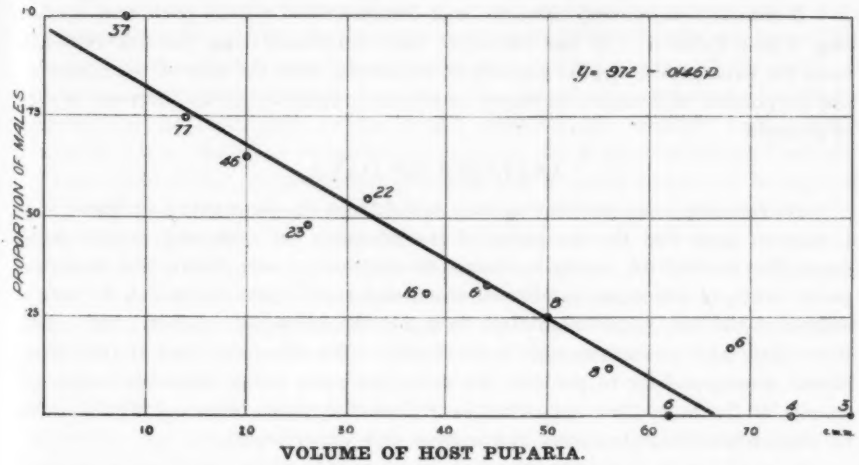

Figure 2. Regression for sex ratio on size of puparia calculated from all puparia for which the sex of the emerging parasite is known. (Numbers indicate the weight of each point).

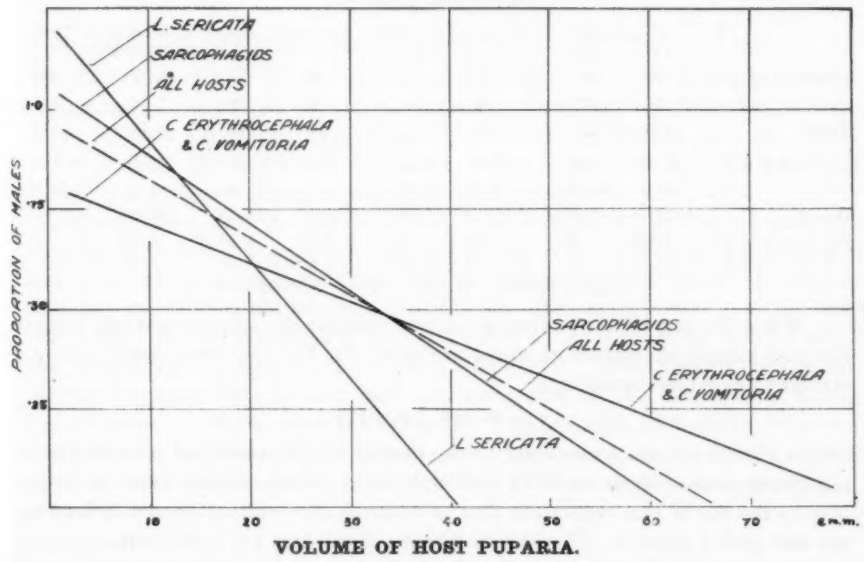

Figure 3. Regression lines for the relation between sex ratio and size of host puparia within each host species. The line for *Calliphora erythrocephala* alone almost coincides with that for all hosts.

A similar relationship has also been demonstrated within each host species (fig. 3 and Table 5). It has therefore been concluded that the different sex ratio for each group of host puparia is connected with the size of the puparia; the proportion of females increases in simple proportion to an increase in size of puparia.

ANALYSIS OF DATA.

By forming a simple contingency table, from the data given in Table 1, it is easy to show that the sex ratios of the parasites for each host sample could not differ, as they do, owing to chance fluctuations alone. Table 3 is a contingency table, in which the calculated values are the frequencies which we would expect if the sex ratio calculated from all the parasites, namely, 208 : 133, were applicable to each sample individually. We conclude from it that if no factor was operating to prevent the same sex ratio being associated with all species of the host, then sex ratios as different as those observed would occur by chance less often than once in a million such experiments.

TABLE 3.

Contingency Table of Sex Ratios arising from Different Samples of Parasitized Puparia.

	L. sericata "1"	*L. sericata* "2"	*C. erythrocephala* "1"	*C. erythrocephala* "2"	*Sarcophaga* spp. "1"	*Sarcophaga* spp. "2"	*C. vomitoria* "1" "2"	Total.
Observed ♂♂	98	74	10	6	3	15	2	208
♀♀	26	43	6	9	9	28	12	133
Total	124	117	16	15	12	43	14	341
Calculated ♂♂	75·6	71·4	9·8	9·1	7·3	26·2	8·5	208·0
♀♀	48·4	45·6	6·2	5·9	4·7	16·8	5·5	133·0
O — C	±22·36	±2·63	±·24	∓3·15	∓4·32	∓11·23	∓6·54	
$\frac{(O-C)^2}{C}$ ♂♂	6·61	·10	·01	1·08	2·55	4·81	5·01	
♀♀	10·34	·15	·01	1·70	3·99	7·52	7·83	

$\chi^2 = 51{\cdot}70 \qquad n = 6 \qquad P = {\cdot}0000005$

When the sex ratio observed is plotted against the mean puparium volume for each sample the values obtained are seen (fig. 1) to lie very closely along a straight line whose equation is

$$y = 0{\cdot}934 - 0{\cdot}0137\ p$$

where y is the sex ratio measured as the proportion of males and p is the size of puparium measured in units of 0·67 cub. mm. There is, however, an objection to the use of this regression line to describe the relation observed between sex and size of puparia. The values for size of puparia are mean values of each host sample, and the range of size shown by a sample overlaps the ranges of adjacent samples. As a result the true interpretation may be obscured. Also it would be difficult, if not impossible, to estimate the errors involved.

The relation between sex and size of puparium would be demonstrated more satisfactorily if a direct correlation could be established between sex of the parasite and size of the puparium irrespective of the species of the host. In order to do this only puparia for which the sex of the respective parasites is known can be used, and, owing to this selection, the samples used are not random. It is therefore necessary to examine the data to determine whether the sex ratio of any particular size group of host puparia appears to be appreciably changed by the discard of those puparia yielding parasites whose sex is unknown (Appendix 1). The data are inadequate to test this question as critically as is desirable. But since no definite evidence to the contrary can be found, it is assumed that the selected sub-samples, comprising only the known sexes have the same sex-size relationship as the whole original samples. Further, batches "1" and "2" are homogeneous, in that they show the same relationship between sex and size of puparia, and so they may be grouped together (Appendix 2). The data have therefore been classified for sex ratio and size of puparia in groups representing six units of puparium volume. Table 2 shows the contribution by each sample of puparia, and also serves to show the range of puparium size covered by each species. From the totals the regression of sex on size of puparium is calculated to be

$$y = 0{\cdot}972 - 0{\cdot}0146\ p.$$

This is shown graphically in fig. 2.

The regression coefficient for the slope of the line (—0·0146) has a standard error of ±0·0015. Since the regression coefficient is 9·7 times its error, it is decidedly significant, and agrees in order of significance with the test in Table 3. (For the degree of association between sex and size of puparia, as measured by the correlation coefficient, *vide* Appendix 3.)

To test how well the line describes the data Table 4 has been constructed. It appears that if this regression represents the true relation between sex and size of puparium a greater deviation from it than has been observed could be expected to occur by chance once in about nine such experiments. At the same time nearly three-quarters of χ^2 is contributed by a single class, that in which the volume of the puparia = 8 units (Table 2). This class is determined entirely by data from *Lucilia*, and an explanation of the discrepancy is suggested below. If the explanation is correct the agreement between the observed and the calculated values is excellent, and indicates within the limits of these observations a simpler linear relation between size of puparia and sex ratio of the parasite.

TABLE 4.

Test of Goodness of Fit for a Straight Regression Line between Sex Ratio and Size of Puparia. (Original Data in Table 2.)

Size of Puparia (Unit=·67 c. mm.)		8	14	20	26	32	38	44	50	56	62	68	74	80
Number of Puparia in each class		37	77	46	23	22	16	6	8	8	6	6	4	3
Proportion of ♂♂ as calculated from the equation y=·972 —·0146 p		·855	·768	·681	·593	·506	·428	·331	·244	·156	·069	-ve	-ve	-ve
Calculated	♂♂	31·64	59·14	31·33	13·64	11·13	6·69			5·60				
	♀♀	5·36	17·86	14·67	9·36	10·87	9·31			35·40				
Observed	♂♂	37	57	30	11	12	5			6				
	♀♀	0	27	16	12	10	11			35				
O — C		±5·36	∓2·14	∓1·33	∓2·64	±·87	∓1·69			±·40				
$\frac{(O-C)^2}{C}$	♂♂	·91	·08	·06	·51	·07	·43			·03				
	♀♀	5·36	·26	·12	·74	·07	·31			·01				

$\chi^2 = 8{\cdot}94$ n = 5 P = ·11

For the relation within species regression lines have been calculated for each species separately. They are shown in Table 5 and represented graphically in fig. 3.

TABLE 5.

The Relation between Size of Puparia and Sex Ratio within Different Host Species (vide Figure 3).

Species.	Regression Equation.	Standard Error of the Coefficient of p.
L. sericata	y = 1·222 — ·0300 p	·0049
C. erythrocephala	y = ·969 — ·0146 p	·0092
C. eryth. and *C. vom.* combined	y = ·810 — ·0097 p	·0040
Sarcophaga spp.	y = 1·057 — ·0172 p	·0033

The line for *Lucilia*, as calculated from the actual data, has a steeper slope than the other lines; the difference between the coefficient for *Lucilia* and *Sarcophaga* is 0·0128 ± 0·0060, and that between those for *Lucilia* and *C. erythrocephala* 0·0154 ± 0·0104. The differences can, however, be explained as due to the discard of certain puparia for which the sex of the emerging parasites could not be determined. Emergence tends to take place earlier from small puparia than from large puparia. Emergence of most of the parasites whose sex could not be linked up with the puparia from which they arose, took place during the first three days of the period in which females emerged. It is therefore probable that there was a tendency for the smallest puparia yielding female parasites to be discarded. If this were the case, the discard of such puparia would explain the steeper slope of the regression calculated for *Lucilia*, and also the deviation of the sex ratio observed for p = 8 from that calculated from the straight line regres-

sion for the whole range. The discard has little effect on the regression for all species together, since only a small range of puparium size is concerned. That the slope might decrease in the region covered by *Calliphora vomitoria* (very large puparia) is to be expected, since there is a physical limit to the sex ratio at $y = 0$. Since all lines cross each other in the centre of the total range, between $p = 15$ and $p = 32$, and since the differences in slope are not definitely significant, it appears reasonable to conclude that each species separately conforms to the relationship shown for all species together.

DISCUSSION.

For an adequate interpretation of the results it would be necessary to decide whether the males were derived from diploid or haploid eggs. Cytological or genetical investigation of sex in this parasite has not yet been made. It is therefore impossible to decide, on the evidence available, from which type of egg the males were derived, whether from haploid eggs laid by unfertilized females, haploid eggs from fertilized females, or from diploid eggs. If they were from haploid eggs derived from *unfertilized* females one would have to conclude that unfertilized females restricted their attention to the smaller hosts. If they were from haploid eggs laid by *fertilized* females one would have to conclude that there was a mechanism for controlling the fertilization of the egg according to the size of the host. From general observations on oviposition by Alysia one would not expect size of host to play a part in this way, for ovipositing females somtimes grip the larvae at the narrow anterior end and sometimes at the thicker posterior end. At other times they are not in contact with the larva in which they are ovipositing; they may be standing either on other larvae or on the skin or flesh of the medium in which the larvae are living, and through which the ovipositor passes to the larva beneath.

A sperm-pump, which regulates the emission of sperms from the spermatheca into the vagina, and hence determines the fertilization or non-fertilization of the eggs as they are laid, has been demonstrated in many of the Aculeate Hymenoptera; but there is no evidence for its existence in the Braconidae. Further, it is evident from the summary of the literature given by Vandel (1931) that, even in those Hymenoptera in which a sperm-pump is known to occur, the generalization usually made regarding a relation between its operation and the size of the receptacle for the egg is far from conclusive. Dzierzon's theory that unfertilized eggs produce drone bees and fertilized eggs produce females (workers or queens), has been confirmed in numerous Hymenoptera. As far as it goes it is supported by evidence from Alysia. But evidence is accumulating that in the Hymenoptera males may be derived from fertilized eggs as well as from unfertilized eggs. Dzierzon himself obtained drone bees with paternal characters (Vandel (1931)). The work of P. W. and A. R. Whiting, followed up by that of Torvik (1931), shows further that biparental males

are common in the Braconidae. Torvik concludes that "the more biparental males are studied the more closely is their genetic composition found to parallel that of diploid females. In spite of this genetic similarity to females, the biparental males are as definitely male in morphology and reaction as are haploid males."

Results similar to those found in Alysia have been recorded from other animals. Malaquin (1901) found that in the Monstrillids, parasitic Copepods which he found as parasites of Annelid worms, if one parasite was present alone its sex was either male or female; but if more parasites than one occurred they were males. Chewyreuv (1913) found that the females of several species of Ichneumonids, when fertilized, produced a preponderance of females if allowed to oviposit in large host larvae, and produced a preponderance of males if allowed to oviposit in small larvae. Christie (1929) found that when the infestation of *Mermis subnigrescens* in grasshoppers was high the worms were male; when low, they were female; and when the infestation was intermediate, both sexes of the parasite occurred. All these cases indicate that when there is a stress on the amount of food available there is a tendency for males to be in excess.

What, then, is the explanation of the correlation between size and sex of Alysia? There is no evidence that the Alysia males were derived from haploid eggs. The circumstantial evidence points to a greater probability of their being derived from diploid than haploid eggs. Should it eventually be shown that males of Alysia are commonly produced by diploid eggs, the most probable conclusion would appear to be that quantity of food, as regulated by size of host, played a part in the determination of sex: that, as Geddes and Thomson (1901) said, "favourable nutritive conditions tend to produce females and unfavourable conditions males." Elsewhere (Holdaway (1930a)) it has been recorded for *Tribolium confusum* that starvation of young larvae for two or three days results in an excess of females being produced. These results, which will be published in detail in the near future, cannot be interpreted on the basis of differential mortality. The conclusion that favourable nutritive conditions tend to produce females and unfavourable conditions males cannot therefore be applied universally.

SUMMARY.

A relation has been established between size of host puparia and sex ratio of the Braconid parasite, *Alysia manducator*. Small puparia produce a high proportion of males, large puparia a high proportion of females. The relation between volume of puparia and sex ratio can be described (within the limits of the observations recorded) by a straight line.

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APPENDIX 1.

Examination of Data to Determine whether the Relation between Sex Ratio and Size of the Puparia is Appreciably Changed by Discarding Puparia to which it is not possible to Assign the Sex of the Parasite Emerging.

In the samples of *Sarcophagids* and *Calliphora* spp. this discarding is not serious, since for only six out of one hundred puparia is the sex of the parasite unknown. But in the samples of *Lucilia* a rather high proportion is unknown. The numbers are shown in Table 6.

TABLE 6.

Number of Lucilia Puparia for which the Sex of the Emerging Alysia is Known or Unknown.

Batch.	Number of Puparia for which the Sex of the Parasite is Known.	Unknown.	Number of each Sex in these Groups. Known. ♂♂	♀♀	Unknown. ♂♂	♀♀
"1"	80	44	66	14	32	12
"2"	88	29	59	29	15	14

The regression for sex ratio on size of puparia calculated from the "known" groups has been applied to the sizes of puparia in the "unknown" groups to estimate the number of each sex we would expect in the latter groups if the regression has not been changed by selection. The regression line used is: $y = 1{\cdot}222 - 0{\cdot}030\ p$. The results are shown in Table 7.

TABLE 7.

Number of Each Sex in the Groups for which the Sex of the Parasite Emerging from each Individual Puparium is Unknown, Compared with Expected Numbers Calculated from Regression of Sex on Puparium Size in the "Known" Groups.

Batch.	"1"		"2"		Total "1" + "2"	
	♂	♀	♂	♀	♂	♀
Observed	32	12	15	14	47	26
Calculated	34·3	9·7	17·2	11·8	51·4	21·6
Deviation	2·3 ± 2·7		2·2 ± 2·6		4·4 ± 3·9	

The error due to selection is not high. Nevertheless the test is far from sensitive, and since too many females relative to the size of the puparia have been discarded in both samples, one suspects that a small systematic error may have been introduced into those selected samples. (*Vide* discussion on Table 5.)

APPENDIX 2.

To Test whether Batches of "1" and "2" are Homogeneous with Respect to the Correlation between Sex Ratio and Size of Puparia.

Since excessively coarse grouping would be required to obtain sufficiently large numbers in all classes in order to apply a χ^2 test, the similarity of batches "1" and "2" has been tested by calculating the regression of sex ratio on size of puparium for each batch separately.

For batch "1" we obtain: $y = 1{\cdot}007 - {\cdot}0145\,p$,
and for batch "2": $y = {\cdot}929 - {\cdot}0140\,p$.

The difference between the slopes of these two lines is $0{\cdot}0005 \pm {\cdot}0031$; and the difference between values of y near the mean ($p = 25$) is $0{\cdot}066 \pm {\cdot}052$. It may therefore be concluded that the two batches show no difference in the relation which exists between sex ratio and size of puparia.

APPENDIX 3.

Analysis of the Relation between Time of Emergence of Parasites, Sex Ratio, and Size of Puparia.

It is difficult to evaluate fully the relation between sex and time of emergence because while emergence of parasites was fairly steady for eighteen days (21st December to 7th January), about one-tenth to one-fourth of each sample emerged at sporadic dates thereafter for two months. The sex ratio of the parasites emerging sporadically is comparable to that of the group as a whole. It therefore appears to be a random sample with respect to sex. For the present study of the relation between sex and time of emergence, only those emerging over the modal period are considered. Adequate numbers for the study occur only in the *Lucilia* groups. By a similar method to that used in Appendix II, batches "1" and "2" have been found to be homogeneous in the relation shown between sex and time of emergence. The emergence of parasites in batches "1" and "2" combined, and in addition the total emergence, is shown in Table 8.

TABLE 8.

Time of Emergence of Parasites from L. sericata, Batches "1" and "2" Combined.

Day of Emergence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19–61	Total.
Males	9	6	5	14	13	23	6	15	14	7	6	8	4	6	9	1	3	4	27	180
Females	–	–	–	–	2	3	1	2	3	9	8	11	0	0	8	5	4	2	11	69

The correlation between sex ratio and time of emergence is, like that between sex ratio and size of puparia, approximately linear, when puparia with delayed emergence (days 19–61) are disregarded. Using puparia for which the sex of the emerging parasites is known, correlation coefficients between the three variables have been calculated. These are of the same order. They are:

$r_{yp} = -\cdot 45$ $\quad r_{yp.t} = -\cdot 31$

$r_{yt} = -\cdot 44$ $\quad r_{yt.p} = -\cdot 29$

$r_{pt} = +\cdot 46$ $\quad r_{pt.y} = +\cdot 33$

$n' = 128$ $\quad P = \cdot 01$ for $r = \cdot 227$

where y = proportion of males

p = size of puparia

t = time measured from the first day of emergence. These coefficients must be interpreted with care since they are calculated from data which have been selected in two ways. They do not show the fundamental connection between the three variates. Either size of puparia or time might be a causal factor in producing the correlation between sex and the other factor, but on the present data, because the partial coefficients are still significant, it appears that time and size of puparium may be to some extent independently connected with sex.

The zero order correlation between sex and size of puparia for all the data considered in Table 2 is $r = -\cdot 51$.